DOCTOR OF PHILOSOPHY

Exploring the relationship between canines, sex and age
A CBCT analysis

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A CBCT analysis

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ABBREVIATIONS

CBCT —Cone beam computed tomography

CT — Computerized tomography

PV —pulp volume

PCV —pulp chamber volume

PTAR —pulp tooth area ratio

PTVR —pulp tooth volume ratio

r—correlation

R^2 —coefficient of determination

SEE —standard error of the estimate

µCT —Micro–computed tomography

2-D —two–dimensional

3-D —three–dimensional

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DEDICATION

I would like to dedicate this thesis to:

My Father to whom I lost in the end of my study. His affection, courage, unconditional love, encouragement made me able to achieve his goal.

My lovely young son, who just joined in the end of my study.

DECLARATION

I, Shakeel Kazmi, declears hat I am the author of his thesis, Exploring the relationship between canines and age. A CBCT analysis, that unless otherwise stated, all references cited have been consulted by myself and the work of which this thesis is a record has been done by myself. Furthermore, I confirm that the work contained within has not been previously accepted for a higher degree at this, or any other institution.

Signed …………………………………Date …………………………….

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ABSTRACT

Radiographic methods using PV and PTVR are important for dental age estimation. According to previous studies, these two markers possess different relationships with age in different sexes but none of the studies have used a homogenous (approximately equal numbers of individuals in each age range) age distribution to assess this relationship and the effect of sex as predictor on age estimation.

This study was performed on 719 subjects of Pakistani origin (368 females and 349 males) aged from 15-65 years. Cone beam computed tomography images of 521 left maxillary and 681 left mandibular canines were collected. Planmeca Romexis® software was used to trace the outline of the pulp cavity and tooth and to calculate respective volumes. Subsequently, Microsoft® Office Excel 2016 was used to calculate the ratios.

Regression analysis was performed to assess the correlation between PV and PTVR factoring sex in as a predictor for age estimation. The obtained results showed that mandibular canine PV (R^2 =0.33) and maxillary PTVR (R^2 =0.46) with sex have the highest predictive power. The relationship between mandibular canine PV and maxillary PTVR with sex against chronological age demonstrates an odd S-shaped non-linear relationship.

The conclusion is that using predictors such as the PV and PTVR with sex produced the best estimates of chronological age.

Chapter 1. Introduction

1.1 Dental age estimation

Teeth are used in forensic, archaeological, and anthropological sciences for estimating the age of living and skeletal remains because they are less affected by the nutritional and environmental factors (Aboshi et al., 2010, Tardivo et al., 2011, Cameriere et al., 2006, Cameriere and Ferrante, 2011). Several methods are available for dental age including morphological, visual analysis of eruption patterns but radiographic methods are commonly used (Kvaal, 2006, Gustafson, 1950, Cameriere et al., 2006, Kvaal et al., 1995). However, each method has its own strengths and limitations, for instance the visual method is based on the time of emergence of the tooth in the oral cavity; once the eruption of the teeth is complete, it is not possible to apply this method for age estimation (Uzuner et al., 2017). Morphological methods such as dentinal transparency, aspartic acid racemization and cemental annulation cannot be used on a living person because they require the extraction /sectioning of the tooth; these methods are also expensive and time consuming (Gustafson, 1950). Radiographic methods are simple, non-invasive, reproducible, feasible, and applicable to both the living and the deceased (Panchbhai, 2011).

Different dental predictors from developing and developed teeth provide opportunities for dental age estimation (Demirjian et al., 1973, Cameriere et al., 2004). The development of teeth is a continuous process, extending from *in utero* to early adult life and consists of number of stages which are widely used with various radiographs as dental age predictors for age estimation (Demirjian et al., 1973, Gleiser and Hunt, 1955, Schour and M.Massler, 1941, AlQahtani et al., 2010). When all teeth have erupted, regressive changes, such as secondary dentine deposition, cementum apposition, transparency of the root, and tooth attrition can be assessed by other methods, most of them are invasive. However, secondary dentine deposition is measurable by assessing radiographs (Kvaal et al., 1995, Cameriere et al., 2006, Gustafson, 1950).

1.2 Age estimation using the visual method

Due to the natural eruption timeline of teeth, the sequence of tooth eruption can be evaluated visually for age estimation. Tooth eruption follows a typical chronological pattern, providing an estimation of age by visual examination of the oral cavity. This method can provide dental age estimation up to 12-13 years old, corresponding to the eruption of the second molars, after which the variability of the third molar eruption makes this a less robust tool. Tooth eruption was used for age estimation in the 19th century in connection to child labour. The Factory Regulation Act of 1833 stated that no child could be employed before the age of 9 and working hours were restricted for children aged 9-13. Variations in physical appearance between children made it difficult to determine their age; therefore, Queen Victoria’s dentist, Edwin Saunders, introduced the concept of visual teeth eruption inspection as a mean to estimate a child’s age (Saunders, 1837).

1.3 Age estimation using secondary dentine as a predictor

Despite having different contents and arrangements, pulp and dentine have a common embryonic origin. These two tissues share a close relationship in terms of physiologic and pathologic reactions. Anything that disturbs the dentine will affect the pulp, and vice versa (Mjor et al., 2001). Once the teeth are fully erupted into the oral cavity, they then undergo various physiological age related changes (Gustafson, 1950). One important age-related change is secondary dentine deposition. Determining when primary dentine has fully formed and secondary dentine formation has begun, however, represents a significant challenge (Karjalainen, 1984). Many investigators believe that root completion represents primary dentine completion and the beginning of secondary dentine formation; whereas, others think tooth eruption as a marker of primary dentine completion and the onset of secondary dentine (Sedgley, 2012, Stanley, 1981, Sloan et al., 2015, Goldberg et al., 2011).

Primary dentine transition into secondary dentine is a age-related factor, not factors related to tooth function (Karjalainen, 1984). It is widely accepted that secondary dentine deposition is a continuous process that decreases the size of the pulp cavity with age. Bodecker first investigated this correlation in 1925 (Bodecker, 1925). However, it was not until 1952, that Gustafson introduced an invasive method using tooth sectioning and reported six age-related changes, including secondary dentine (Gustafson, 1950).

1.4 Age estimation using teeth

Teeth are preferred in age estimation methods because they are less influenced by nutritional, hormonal, and environmental factors than is bone (Ugur Aydin and Bayrak, 2018, Aboshi et al., 2010, Tardivo et al., 2011). Although different teeth have been proposed as useful for age estimation, some researchers prefer to use canines because of the following reasons (Adisen et al., 2018, Tardivo et al., 2014, Jagannathan et al., 2011, Gulsahi et al., 2018, De Angelis et al., 2015, Tardivo et al., 2011, Star et al., 2011, Lee et al., 2017b, Yang et al., 2006, Biuki et al., 2017, Sasaki and Kondo, 2014, Vandevoort et al., 2004, Ge et al., 2016):

- Due to their location in the mouth, canines are often termed as cornerstones, and so are less affected by periodontal diseases and occlusal stress.

- Canines have a high survival rate in dentition. They are often the last teeth that remain in the mouth and are most frequently found in old age and historical populations.
- Canines have larger dimensions and bigger pulps than other teeth, which makes measurement on radiographs easier.
- Canines undergo less wear than anterior and posterior teeth because of their specific function.

1.5 A brief history of forensic radiology

On 8 November 1895, Wilhelm Röntgen accidentally discovered image-producing rays. The nature of the rays was unknown to him, therefore, he named them ‘X-rays’. Soon after this discovery, in 1896, Dr Otto Walkhoff, recorded his own full-mouth dental roentgenogram, introducing ‘X-rays’ into dentistry (Shah et al., 2014). In the same year, Professor Arthur Schuster introduced the earliest application of radiology for forensic use to study the location of four bullets in a shooting victim’s head (Eckert and Garland, 1984). Dr Oscar Amoedo documented the first case of dental age estimation in 1898, in his book *“L’Art Dentaire en Médicine Légale”* (Lichtenstein, 1996).

In the 1940s, dental radiology applications were introduced into the field when forensic dentists were involved in identifying the victims of an aircraft crash in Scandinavia (Lichtenstein, 1996). Since then, radiology has become an important tool in forensic sciences for various purposes, such as human identification and age estimation (Sivaneri et al., 2018, Cameriere et al., 2006).

In forensic odontology, radiographic images and dental age predictors from developing and developed teeth provide a non-invasive approach for estimating dental age (Uzuner et al., 2017). In addition, various methods have been developed using combinations of radiological images and predictors for age estimation (Kvaal et al., 1995, Cameriere et al., 2004). The application of methods for age estimation depends on the identification task, the availability of the apparatus, and the images stored in the archives.

1.6 Age estimation using radiographic methods

Over time, the scope of forensic radiology has increased. Forensic dental radiology is widely used to identify deceased individuals and to estimate age. The ante- and post-mortem dental radiographs are the most accurate means of identifying victims (Manigandan et al., 2015). For age estimation, dental methods can be categorised into two approaches:

- Methods using radiographs of developing teeth
- Methods using radiographs of developed teeth

For decades, the visual method was the only method for estimating age. Later, the patterns of tooth development and eruption on dental radiographs were considered a reliable indicator (Moorrees et al., 1963, Demirjian et al., 1973, Gleiser and Hunt, 1955, AlQahtani et al., 2010). These methods divide tooth development into 8, 10, 17, and 22 stages (Demirjian et al., 1973, Nolla, 1960, Gleiser and Hunt, 1955, Schour and M.Massler, 1941). These stages provide age estimation either by one of two methods, either the atlas method or the scoring method. (Schour and M.Massler, 1941, Nolla, 1960).

The atlas method consists of a series of drawings of tooth development and eruption with age. A developing tooth radiograph is matched with the illustrations, and the corresponding age can be estimated (Moorrees et al., 1963). In contrast, the scoring method consists of scores assigned against the developing tooth. The development of the tooth is divided into different stages, and these are awarded different scores. The scores are added and matched to a table that provides the estimated age (Demirjian et al., 1973). Both methods are simple, non-invasive, reliable, and reproducible. These methods have disadvantages also, including the lack of even distribution of age and small sample size, and they fail to cover the entire developing dentition. To overcome these limitations and to develop a new more comprehensive method, The London Atlas of human Tooth Development and Eruption was produced (AlQahtani et al., 2010). This version provides age estimation from 28 weeks *in utero* to 23 years old and presents sex neutral drawings of tooth development, and the midpoint of the age category is introduced instead of age ranges. The performance of the London Atlas and previously developed methods suggest that all methods tend to underestimate age, but the London Atlas is closest to the chronological age (AlQahtani et al., 2014b). In addition, a large sample size with uniform age distribution reduces the variations and comprehensively explains the understanding of the age estimation.

The development of the permanent dentition completes with the eruption of the third molars, usually between 17 and 21 years old. After this time, radiographic age estimation based on developing teeth is impossible. However, other methods based on age-related change, such as secondary dentine formation, provide further opportunities to estimate age. Once tooth development is complete, odontoblasts begin continuous production of secondary dentine against the pupal walls (Kawashima and Okiji, 2016). Kvaal et al. and Cameriere et al. are examples of authors who assessed secondary dentine in their methodologies for estimating age (Kvaal et al., 1995, Cameriere et al., 2004).

1.7 Two–dimensional radiographic studies

Periapical and panoramic radiographs are the most commonly used radiographs in dental practice; they provide a two–dimensional (2–D) image of a three–dimensional (3–D) object. Their primary aim is to provide insights into the tooth structure to supplement the clinical examination. Kvaal et al. and Cameriere et al. used the findings of Bodecker and Gustafson, to measure the amount of secondary dentine formation and used it as a predictor in age estimation in their respective methodologies (Kvaal et al., 1995, Cameriere et al., 2004, Bodecker, 1925, Gustafson, 1950).

Kvaal et al. introduced a combined method based on radiological and morphological measurements. The pulp and tooth length and width were measured and converted into ratio to can be used as dental age predictors (Kvaal et al., 1995).

The measurements from the Kvaal et al. methodology reveal a high degree of intra- and inter class correlation, indicating the reproducibility of the technique and the degree of agreement amongst raters. Therefore, many studies tested this approach and used it in different populations for age estimation (Marroquin et al., 2017, Bosmans et al., 2005, Kvaal et al., 1995, Hisham et al., 2019, Misirlioglu et al., 2014, Erbudak et al., 2012, Marroquin Penaloza et al., 2016, Landa et al., 2009, Meinl et al., 2007, Karkhanis et al., 2014, Roh et al., 2018, Akay et al., 2019, Paewinsky et al., 2005, Willems et al., 2002, Mittal et al., 2016). The Kvaal et al. study produced a 0.76 coefficient of determination (R^2) with ±8.6 years of standard error of the estimate (SEE) but the best results (SD =5.6, r = -0.95) were obtained from the German population by combining the width ratios from all teeth from panoramic radiographs using Hipax software (Paewinsky et al., 2005).

Sex was not used as a predictor in some studies but compared the linear measurements of pulp tooth area ratio (PTAR) between males and females. Mixed results were found, as some studies report that no difference was found between males and females (Shetty et al., 2010, Paewinsky et al., 2005, Saxena, 2011). Whereas, some suggest that differences did exist (García et al., 2009, Ayad et al., 2014).

Cameriere et al. introduced a new method for age estimation using PTAR in periapical radiographs of canines. Like the Kvaal et al. method, the Cameriere et al. method also reported a high degree of intra- and interclass correlation, indicating the reproducibility of the technique. Initially, the Cameriere et al. method was designed for canines, but was subsequently applied to other teeth (Cameriere et al., 2013, Cameriere et al., 2012).

Most of the studies did not use sex as a predictor but report that the sex and PTAR does not show any correlation; thus, sex was excluded from the regression analysis (Cameriere et al., 2004, Cameriere et al., 2007b, Cameriere et al., 2009, Cameriere et al., 2012, Babshet et al., 2010). However, a few studies report that sex significantly correlates with PTAR and, thus, included in the regression models for analysis (Cameriere et al., 2013, Ravindra et al., 2015, Sakhdari et al., 2015).

1.8 Three–dimensional radiographic studies

Two–dimensional (2–D) images provide excellent images for dental diagnostic needs but, unfortunately, the superimposition of structures leads to an inability to assess the pulp structure and recognition of the overall shape of the tooth accurately (Shah et al., 2014). In other words, these radiographs provide 2–D images of 3–D objects (Whaites and Drage, 2013). However, 3–D objects must be visualized from several positions. The first computerized tomography (CT) scanner was developed in 1972 to overcome these limitations (Shah et al., 2014). Later, cone beam computed tomography (CBCT) was added to the dental radiology for dental diagnostics.

In recent years, 3–D imaging has become a diagnostic adjunct for oral and maxillofacial surgery, endodontics, implantology, and orthodontics. These images are mostly composed of CBCT which consists of axial, sagittal, and coronal images, which provide a visualisation of a tooth from three different perspectives. Thus, highly detailed information of a tooth can be achieved. Using these images, researchers have calculated the pulp volumes (PV) and the ratio of pulp tooth volume ratio (PTVR) and utilised them for age estimation (Ge et al., 2015, Vandevoort et al., 2004).

In 2004, Saka et al. first observed the morphological changes in maxillary first premolars using µCT. A decrease in the shape and PV with age was noticed (Oi et al., 2004). Similarly, in the same year, Vandevoort et al. calculated the volumes of pulp and tooth using µCT, and PTVR were correlated with age. A coefficient of determination (R^2) of 0.31, was found between PTVR and age but promising results achieved for estimating age based on PTVR (Vandevoort et al., 2004).

Another study, correlated linear ratios from first and second premolar teeth and suggested that the coronal one-third of the root provides the best correlation with age, and that the apical one-third of the root is the worst (Aboshi et al., 2010). Another study utilised a large sample of mandibular canine pulp root volume ratio, and the results suggest that pulp root volume ratio is a useful indicator for estimating age, especially if the sex is known to be female (Sasaki and Kondo, 2014).

Yang et al. were the first to utilise CBCT on 81 single-rooted teeth to assess the correlation between PTVR and chronological age using specially developed software.

Results showed a moderate correlation (R^2=0.29) between PTVR and age (Yang et al., 2006). Similarly, 0.32 and 0.38 coefficient of determinations were found by evaluating a small sample of canines between PTVR and age (Jagannathan et al., 2011, Tardivo et al., 2011). In addition, Angelis et al. performed CBCT on 91 maxillary canines and calculated the ratio from pulp chamber tooth volumes and a 0.38 coefficient of determination was achieved (De Angelis et al., 2015). However, lower coefficients of determinations of 0.07 and 0.23 were found in some studies utilising PTVR, possibly attributable to a small sample size (Star et al., 2011). A study based on the CT scans of 133 PTVR of canines reported a moderate (0.38) coefficient of determination (Tardivo et al., 2011). Tardivo et al. studied 840 canines CT scans and found that maxillary and mandibular PTVR are very useful in age estimation (Tardivo et al., 2014).

Regarding the relationship between PTVR and age, the majority of studies with small to moderate sample sizes reported a linear relationship (Asif et al., 2018, Sakuma et al., 2013, Biuki et al., 2017, Star et al., 2011, Ugur Aydin and Bayrak, 2018, Yang et al., 2006, Vandevoort et al., 2004, Someda et al., 2009, Gulsahi et al., 2018, Haghanifar et al., 2019, De Angelis et al., 2015, Tardivo et al., 2011). However, studies with a moderate to large sample size reported a non-linear relationship between PTVR and age (Tardivo et al., 2014, Sasaki and Kondo, 2014).

Previous 3–D studies evaluating PTVR differences between males and females observed inconsistent results. Several studies report no significant difference between PTVR and sexes (Yang et al., 2006, Gulsahi et al., 2018, Tardivo et al., 2014, Vandevoort et al., 2004, Pinchi et al., 2015, Jagannathan et al., 2011). Despite of no difference, some studies report that PTVR of females showed stronger association with age (Star et al., 2011, Agematsu et al., 2010, Sasaki and Kondo, 2014). Whereas, some found that the PTVR of males has stronger relationship with age (Asif et al., 2019, Sakuma et al., 2013, Tardivo et al., 2011).

In contrast to above findings, however some studies found significant difference between PTVR and sexes. Regardless of difference, some studies reported that PTVR of females has stronger association with age (De Angelis et al., 2015, Someda et al., 2009). Conversely, some studies found that PTVR of males more closely associated with age (Biuki et al., 2017, Haghanifar et al., 2019).

More recently, a new method based on calculating only PV was introduced into 3–D studies for estimating age. A moderate sample of molars was used to investigate the pulp chamber volume (PCV) correlated with age. The results suggested that PCV of first molar is a useful index for estimating age (Ge et al., 2015). Another study by Ge et al. sought the best relationship between age and PV in 13 types of teeth. The results indicate that maxillary second molars displayed the highest correlation with age (Ge et al., 2016). These findings suggest that PV alone can be useful for age estimation (Ge et al., 2015, Ge et al., 2016, Sue et al., 2018).

In relation with PV and age, non-linear and quadratic relationships were found. Regarding PV differences among sexes, one study found a significant difference in PV between males and females. In addition, a stronger relationship was found between PV and age in females than with males (Ge et al., 2015). Another study reports a significant difference in PV and sex in 13 types of teeth except for the mandibular first molar (Ge et al., 2016).

Age estimation research is highly affected by the number of individuals in each group and the selected age range (Biuki et al., 2017, Bocquet-Appel and Masset, 1982). These studies have some common limitations, such as lack of uniform age distribution, small sample sizes, and a failure to cover maximum dentition. Therefore, a large sample size characterized by a homogenous age distribution is a sensible modification to understand age estimation better using CBCT technique. In addition, these factors will provide a better understanding of the relationships of PV and PTVR with age and sex, which will be helpful for age estimation.

Chapter 2. Age estimation using Teeth

In forensic dentistry, PV, PTAR, and PTVR from teeth provide an opportunity to estimate the age. PV and PTVR are measured from 3–D while PTAR from 2–D images. In 3–D images, µCT and CBCT are most commonly used to measure the pulp and tooth volumes whereas, periapical and OPGs radiographs are employed in 2–D images. 3–D and 2–D images quantify morphological changes related to age such as secondary dentine, thus provides a non-invasive approach to estimate the age.

2.1 Age estimation using pulp volume

PV is the recently introduced method to estimate age in adults. This method is a non-invasive approach based on the formation of secondary dentine and the decrease of the pulp size with age. 3–D imaging has been introduced into the dentistry in the early 20th century for diagnosis and treatment planning. However, introduction of 3–D imaging have provide great opportunity to evaluate the age related 3–D changes in pulp cavities.

2.1.1 Three–dimensional pulp changes with age

In 2004, Oi et al. used µ-CT scans of the extracted maxillary first premolar to observe the PV changes with age and compared PV at a specific site in young, middle and old age. Five points were placed at an equal distance to each other, perpendicular to the pulp length from root apex to the pulp chamber. A line was passed between these points to establish the regions of interest in the pulp chamber. One more line was passed at the pulp chamber floor, to create two artificial regions for the volumetric measurements. These two regions were named the horn and floor regions of the pulp chamber according to their locations. The volumes of the horn and floor regions, and the pulp chamber itself were measured and compared between young, middle and old age groups. In addition, visible findings noted in these regions and root orifice diameters were observed from the root apical direction for the volume changes between young, middle and old age groups. An analysis of covariance (ANCOVA) with Fisher's Protected Least Significant Difference (PLSD) test was used to assess the statistically significant differences between the zones and orifices in the young and old ages. The regions of interest are illustrated in (Figure 3.1). Revised from Oi T et al. (International Endodontic Journal 2004; 37:1: 46-51).

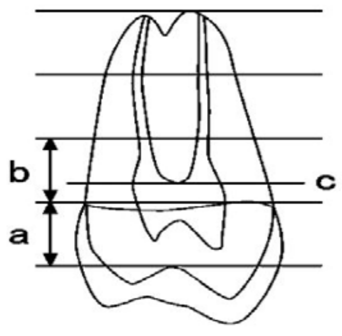


Figure 2.1 The regions of interest in the maxillary first premolar (a) horn region (b) floor region (c) Floor of the pulp chamber.

The results from the horn region of the pulp chamber revealed that the mesial-distal width and height were the largest in the young age and reduced with age. Additionally, the shape of the pulp horn changed and become rounder with age. Similarly, the results of the floor region of the pulp chamber found that the bucco-lingual widths of both canals were largest in the young age and reduced in old age. Furthermore, the shape of the furcation region altered from a V shape to a U shape with age. In terms of PV with age, the volume decreased in all three regions with age. Moreover, significant differences ($p<0.05$) were noted in all regions of PV with age. A major reduction occurred between the 20 years old and 40 years old compared with the 40 years old and 60 years old age groups. This reduction could be due to transition of the primary dentine into secondary dentine as tooth comes into functional state.

Similarly, changes in the orifices of the pulp indicate the same trend in the regions of pulp with age and in different age groups. Moreover, no difference was found between the diameter of the orifices of the buccal and lingual roots compared with age.

In conclusion, despite a small sample used for the analysis, the study indicates that PV changed with age. Furthermore, the volume of the pulp cavity is strongly related to age (Oi et al., 2004).

2.1.2 Pulp chamber volume for age estimation

Ge et al. utilised CBCT images of maxillary and mandibular first molars to calculate the PCV and then used these as a predictor for age estimation. PCV was selected due to the molar complex root system. Regression analysis was carried out between calculated PCV and age to assess the relationship between them. For age estimation, separate equations were formed for the sexes.

The results reveal a statistically significant difference (p=0.013) in PCV between males and females and so was the statistically significantly difference (p=0.028) noticed between the PCV of the maxillary and mandibular first molars. Coefficient of determinations 0.684 and 0.612 were obtained from the maxillary and mandibular first molars, respectively, from females whereas, the PCV of the maxillary and mandibular first molars of males provided 0.544 and 0.562 coefficient of determinations respectively. These results indicate a higher predictor power for females regarding PCV for age estimation.

Using, PCV of all first molars for age estimation produced interesting results. The results reveal that the difference between the estimated and chronological ages was lowest in young adults; the difference was static in middle age and increased in old age. About 10 year difference was found between the estimated and chronological age in the 50 years old and 60 years old age groups. This observed difference could be attributed to sample size, as a relatively small number of teeth were included in the older age groups. In old age, people are more prone to caries and loss of teeth, thus, it was difficult to obtain unaffected teeth for analysis. Another possible explanation for this difference might be an unremarkable decrease in PV in old age. Further analysis found no significant difference (p=0.254) between any of the teeth PCV of the 50 years old and 60 years old age groups (Ge et al., 2015).

Regarding the relationship, a non-linear relationship between all molars PCV and age was found. A sharp decline is apparently noticed in the PV of younger age groups which later slows and becomes stable from middle age to old age (Ge et al., 2015).

Similarly, Sue et al. utilised CBCT images of maxillary and mandibular first molars to assess the relationship between PCV and age. Their sample was divided into younger, middle and old ages to observe the volume changes in these three groups (Sue et al., 2018).

The mean PCV of maxillary molar was larger and displayed significant difference then mandibular molar between young (p=0.000), middle (p=0.000), but was not significant in old (p=0.094) ages.

The PCV of molars and age showed that linear relationship tried to fit in. This result might be due to the uneven distribution of the sample size, as most of the images were selected form the younger age. Despite the uneven distribution of data, the results suggested that PV decreased with age and can be useful in age estimation.

Regression analysis results provided coefficient of determination values of 0.586 and 0.609 from the maxillary and mandibular first molars PCV against age (Sue et al., 2018). These results agree with the findings of Ge at al., in which the mandibular first molar PCV is strongly correlated with age when compared with the maxillary first molar PCV (Ge et al., 2015). However, these results differ from another study by Ge et al., in which the maxillary first molar PCV strongly correlated with age than the mandibular first molar PCV (Ge et al., 2016).

2.1.3 Identification of suitable pulp chamber volume

Ge at al. aimed to determine which tooth PV has the strongest relationship with age and identified whether using single or multiple teeth PV improved the age estimation. For this purpose, the PV of all the teeth was investigated, except maxillary first premolars and third molars. The difference between the PV of males and females was also evaluated.

The CBCT images of 0.15 mm voxel size and ITK-SNAP 2.4 software were used for PV measurements. For single-rooted teeth, the whole PV was calculated but, for multi- rooted teeth, the PCV were included because of the complex root morphology.

The results of all the teeth PV measurements reveal that a difference was found between males and females except in mandibular first molar ($p=0.102$). The maxillary second molar displayed the strongest correlation, and the maxillary canine displayed the least correlation in males, females, and a combined pool with age.

Regarding age estimation, maxillary second molar produced the least difference between estimated and chronological ages. Conversely, the combination of selective PV improved the coefficient determination and reduced the difference between the estimated and chronological ages.

A non-linear relationship between the maxillary second molar PCV and age was found. A sharp incline was notice in the PV at a young age which eventually becomes slow and stable in old age.

Overall, the study has gone some way towards enhancing the understanding of the PV of teeth with age, but the authors suggest that further research is required using a large and homogeneous (approximately equal number of individuals in each age range) distribution sample (Ge et al., 2016).

2.1.4 Age and Sex estimation using pulp volume

Andrade et al. attempted to develop and validate the formula for age and sex estimation using the PV of maxillary central incisors and canines. For this purpose, CBCT images of voxel size 0.2 and 0.3 mm were selected to measure the PV of these teeth. The resultants mean PV was used to form regression equations for age and sex estimations. The equations were derived using the PV of maxillary central incisors, canines, and both of these teeth with and without sex as a predictor.

The relationship between PV and age were determined using Pearson correlation. The results indicate a negative and significant relationship with a Pearson correlation coefficient of ($p<0.0001$).

The results of age estimation using the combination of maxillary central incisor PV along sex displayed a significant relationship with age. Male maxillary central incisor PV produced better age estimation than female PV. Similarly, age estimation results using maxillary canine PV were also significant with age, but the best results were produced using known sex. The maxillary canine PV of females produced better results than males PV.

Using a combination of both teeth PV, the best results were again produced using the known sex. The PV of both teeth in both sexes produced a high coefficient determination but the PV of females ($R^2=0.8341$) produced marginally better results than males PV ($R^2=0.8255$). These results indicate that including sex as a predictor improves the age estimation results.

The results of the sex estimation formulas indicated that knowing the age produced better results than not knowing the age in all three PV categories. However, the combination of both teeth PV produced better results than using a single tooth PV.

The PV of the independent sample was used to estimate the age from the obtained formula. Validation results showed that best age estimation results were obtained when sex was known. The lowest SEE values were found for females as compared to males. Regarding PV of teeth, maxillary canines produced better age estimation than maxillary central incisors.

The PV of the validation sample was also used in the sex estimation formulas. The estimated sex was compared to the actual sex. Results showed that high accuracy was found when age was known. Additionally, PV of combination of a maxillary central incisor and canine produced better sex estimation results than individual PV.

Validation results indicate that age is overestimated up to the age of 35 years old. After this age, there is very good agreement between the estimated and chronological ages, irrespective of whether the sex known (Andrade et al., 2019).

Although, one scan of each sex from 13 to 70 years old was selected, a linear relationship tried to fit in between PV and age. In general, better age estimation results were obtained for individuals who were older than 35 and females (Andrade et al., 2019).

All these studies produced conflicting results regarding PV and its relationship with age and in the different sexes (Ge et al., 2015, Ge et al., 2016, Andrade et al., 2019). However, these existing studies had different sample sizes. No previous study provides information about PV and age, nor PV differences between the sexes using a large sample size. Therefore, this present study, for the first time, explores, and contributes to an understanding of PV and age, as well as PV differences between the sexes using a large homogenous (approximately equal number of individuals in each age range) sample.

2.1.5 Summary of the age estimation using pulp volume

- Mandibular PCV is larger than maxillary PCV.
- A rapid reduction is noticed in the PV of teeth from young to middle age. Slow and stable reduction in PV is noted in middle to old age.
- Regarding relationship between PV and age, mostly studies found a non-linear relationship between them.
- In terms of sex, PCV of females showed better correlation with age as compared to males.

2.2 Age estimation using pulp tooth area and volume ratio

This chapter describes the relationship between pulp tooth area/volume ratios and age and the role of sex as a predictor in age estimation. There are many methods available for age estimation in forensic odontology, but methods relying on teeth and bones are commonly used for age estimation (Uzuner et al., 2017, Schmeling et al., 2016, AlQahtani et al., 2014a, Greulich and Pyle, 1959). Teeth are preferred to bone because they have the benefit of longer preservation, being more durable and have more resistance to destruction than bone over time (Someda et al., 2009). In forensic odontology, age estimation can be performed using various methods (Gustafson, 1950, Kvaal et al., 1995, Cameriere et al., 2004). Among these methods, secondary dentine deposition measurement via radiographs is a non-destructive method (Kvaal et al., 1995, Cameriere et al., 2004).

Pulp and teeth are measured on different radiographs and converted into a pulp tooth area or volume ratio for use as a predictor of age estimation (Cameriere et al., 2004, Kvaal et al., 1995). Pulp and tooth behave differently over time as pulp size reduces; whereas, teeth are less affected with age. Therefore, researchers use a ratio of these two tissues as a predictor for estimating age. The ratio was chosen to diminish the magnification and angulation issues associated with radiographs and to overcome variations related with tooth morphology, size and in different populations (Kvaal et al., 1995, Star et al., 2011).

Several studies have reported different strengths of correlation, with linear and non-linear relationships between the PTVR of teeth and chronological age in different populations. The majority of studies used a small sample and found a linear relationship between PTVR and age (Vandevoort et al., 2004, Someda et al., 2009, Aboshi et al., 2010, Yang et al., 2006, Star et al., 2011, Gulsahi et al., 2018, Biuki et al., 2017, Haghanifar et al., 2019, Asif et al., 2018, Sakuma et al., 2013, Asif et al., 2019, Porto et al., 2015, Ugur

Aydin and Bayrak, 2018). However, some studies used medium and large samples and reported a non-linear relationship between PTVR and age (Tardivo et al., 2014, Sasaki and Kondo, 2014). Previous studies have used a limited age range that failed to cover the entire life period, restricted the sample to small age range, or used an insufficient sample size. Additionally, common drawbacks of previous studies are a lack of uniform age distribution, a homogenous sample size (approximately an equal number of individuals in each range) and an absence of sex as a predictor to describe the relationship of the PTVR with age.

When estimating age, the majority of studies did not find any difference between the PTVR of females and males (Vandevoort et al., 2004, Star et al., 2011, Tardivo et al., 2014, Jagannathan et al., 2011, Misirlioglu et al., 2014, Sakuma et al., 2013, Tardivo et al., 2011, De Angelis et al., 2015). However, a few studies reported that female PTVR produced more accurate results in age estimation than male PTVR (Someda et al., 2009, Porto et al., 2015, Agematsu et al., 2010). Thus, in this study a comprehensive homogenous sample size by age was used to explore the relationship between PTVR and age. Furthermore, the sex differences were tested as predictors for fabricating models to assess their role in age estimation.

2.2.1 Age estimation using pulp tooth area ratio

Conventional dental radiographs compress the 3–D anatomy of a tooth into a 2–D image. In other words, images acquired using 2–D radiology reveal height and width, but the third dimension (depth) is limited (Patel et al., 2009). Conventional dental radiographs require prolonged time and chemical solutions for image development, as well as a darkroom for handling radiographs. All these disadvantages have been overcome with the advent of digital radiographs, but the amount of information in the third dimension (depth) remains limited (Shah et al., 2014, Patel et al., 2009).

The applications of periapical and panoramic radiographs are different from each other. Periapical radiographs are used to evaluate the tooth and surrounding area; - whereas, panoramic radiographs provide the entire dentition, in a single image (Shah et al., 2014). These 2–D radiographs are commonly used in dentistry to support the diagnosis. Subsequently, these 2–D radiographs are used in forensic dentistry for age estimation. Using these 2–D radiographs Kvaal et al. and Cameriere et al. generated their own methodologies for age estimation (Kvaal et al., 1995, Cameriere et al., 2004). Additionally, their work provides an opportunity to assess the use of 2–D radiographs age estimation.

2.2.1.1 Kvaal et al. method

In 1995, Kvaal et al. introduced a method to estimate age in adults. To find suitable predictors for age estimation, the lengths and widths of both pulp and teeth were measured at three defined levels, then converted into Kvaal et al. dental ratios and correlated with age (Kvaal et al., 1995).

Periapical radiographs of maxillary central, lateral incisors, second premolars, mandibular lateral incisors, canines and first premolar were obtained using the parallel technique were selected for linear measurements. A Vernier calliper was used to measure three maximum lengths on the mesial surface of the teeth, pulp, and roots whereas, a stereomicroscope with a measuring eyepiece was used to measure three widths of the pulp and roots. The description of measurements, according to Kvaal et al., is in (Figure 2.2) and (Table 2.1) (diagram is adapted from kvaal et al. from Forensic Sci Int. 1995; 74(3): 175-85)

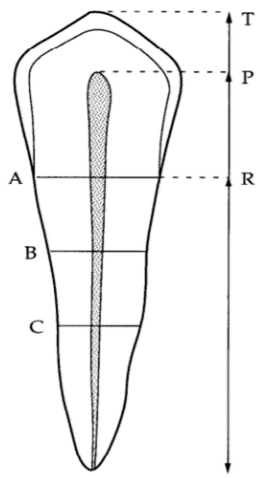


Figure 2.2 Diagram of the measurements according to the Kvaal et al.

Table 2.1 Measurements of length and width with notation and description.

Measurements	Notation	Description
Maximum tooth length	T	Distance from tooth tip to root apex
Maximum pulp length	P	Distance from pulp horn to root apex
Maximum root length	R	Distance from the mesial aspect of CEJ junction to root apex
Level A	A	Width of the pulp and root at CEJ junction
Level B	B	Width of the pulp and root in midway between Level A and C
Level C	C	Width of the pulp and root in midway between CEJ and apex

Mean values of the linear measurements of the pulp and teeth were utilised to develop the 'seven Kvaal et al. dental ratios'. Descriptions of the seven Kvaal et al. ratios are described in (Table 2.2). The ratios were selected to compensate for the differences related to the magnification and angulation of the radiographs.

Table 2.2 Measurements of length and width with notation and description.

Ratios with descriptions	Notation
Ratio between length of pulp and root	P
Ratio between length of tooth and root	T
Ratio between length of pulp and tooth	R
Ratio between width of pulp and root at enamel-cementum junction	A
Ratio between width of pulp and root at midpoint between level C and A	B
Ratio between width of pulp and root at mid-level	C
Mean values of all ratios except ratio T	M

Suitable variables were selected in the stepwise procedure. First, the Pearson correlation coefficient was carried out to assess the relationship between the seven Kvaal et al. dental ratios (Table 2.2) and age. The results reveal that all the ratios were negative and significantly correlated with age, except for Ratio T. However, Ratio M displayed the highest and most consistent correlation value for all the teeth; therefore, it was selected as a predictor for age estimation.

The remaining five ratios of P, R, A, B and C were selected for further correlation analysis to select suitable predictors for age estimation. The rest of the Kvaal et al. dental ratios reveal mixed correlation values therefore mean value of ratios from Levels B, C, P and R selected for further analysis. Since the obtained correlation values of the P, R, C, and B ratios are closely related to each other, the means and difference of the four ratios were correlated with age to find the most correlated ratio as a predictor for age estimation. The correlation analysis results suggest that Ratio W-L was most strongly correlated with age, so it was selected as a second predictor for age estimation. Descriptions of the ratios are provided in (Table 2.3). Description of the Kvaal at al. dental ratios and notations.

Table 2.3 Description of the Kvaal at al. dental ratios and notations.

Ratios with descriptions	**Notations**
W	Mean value of ratios from Levels B and C
L	Mean values of length P and R ratios
W-L	Difference between W and L ratios

For age estimation, six regression equations were derived. The output of the regression equations was expressed in a coefficient of determination (R^2), and the difference between estimated and real age was calculated in a standard error of estimate (SEE). Additionally, values of predictors were used separately in the regression formula for mandibular lateral incisors to find the difference between the males and females (Table 2.4).

Table 2.4 Individual and multiple regression models. G = gender: male=1 female=0.

Teeth	**Equation**	R^2	**SEE (years)**
11/21	Age = 110.2 – 201.4 (M) – 31.3 (W-L)	0.70	9.5
12/22	Age = 103.5 – 216.6 (M) – 46.6 (W-L)	0.67	10.0
15/25	Age = 125.3 – 288.5 (M) – 46.3 (W-L)	0.60	11.0
34/44	Age = 133.0 – 318.3 (M) – 65.0 (W-L)	0.64	10.5
33/43	Age = 158.8 – 255.7 (M)	0.56	11.5
32/42	Age = 106.6 – 255.7 (M) – 61.2 (W-L) – 6.0 (G)	0.57	11.5
All six teeth	Age = 129.8 – 316.4 (M) – 66.8 (W-L)	0.76	8.6
Three maxillary teeth	Age = 120.0 – 256.6 (M) – 45.3 (W-L)	0.74	8.9
Three mandibular teeth	Age = 135.3 – 356.8 (M) – 82.5 (W-L)	0.71	9.4

The individual regression model results indicate that the best outcome for age estimation was produced from the maxillary central incisor (R^2= 0.70) (SEE ± 9.5 years) followed by the maxillary lateral incisor (R^2= 0.67, SEE ± 9.5). Conversely, the mandibular canine

(R^2= 0.56, SEE ± 11.5) produced the least accurate results for age estimation. This discrepancy of a low result (R^2= 0.56, SEE ± 11.5) from the mandibular canine could be attributed to using a single predictor. Overall, regression model results using multiple predictors from combined six teeth (R^2= 0.74, SEE ± 8.6) improved the age estimation accuracy and had a high correlation as compared from single teeth.

The results of the Kvaal et al. study suggest that a strong correlation exists between the dental ratios and age. Similarly, the difference obtained from estimated and chronological ages was lower when using results from combined teeth than single teeth. The effect of sex as a predictor was assessed in the regression formula of mandibular lateral incisors. The results reveal that six years of difference was found between males and females, and that males were more strongly correlated with age.

The full procedure of measurements is quite lengthy and time consuming. Measurements and ratios used the same notations, which makes reading the methodology quite complicated. Ultimately, no particular reason was provided for why these six teeth were selected for the research. Kvaal et al. recommend testing their methodology in different populations, and it has been applied worldwide (Table 2.5).

Table 2.5 Studies reporting the use of the Kvaal et al. methodology.

Author	Year	Country	Age (years)	Total (n)	Measuring tool	Radiograph
Kvaal et al.	1995	Norway	20-87	100	Vernier calliper and stereomicroscope	Periapical
Paewinsky et al.	2005	Germany	14-81	168	Hipax Software	OPG
Bosman et al.	2005	Belgium	19-75	197	Adobe Photoshop 6	OPG
Meinl et al.	2007	Austria	13-24	44	Adobe Photoshop 6	OPG
Landa et al.	2009	Spain	14-60	100	Image J	OPG
Sharma et al.	2010	India	15-60	100	Trophy RVG	Periapical
Saxena et al.	2011	India	21-60	120	Auto CAD 2005	OPG
Erbudak et al.	2012	Turkey	14-57	123	Image J	OPG
Talreja et al.	2012	India	25-77	100	Adobe Photoshop	Periapical
Limdiwala et al.	2013	India	20-55	150	Kodak dental imaging	OPG
Karkhanis et al.	2014	Australia	20-73	279	Image J	OPG
Misirlioglu et al.	2014	Turkey	17-72	114	Adobe Photoshop	OPG
Mittal et al.	2016	India	14-60	152	VistaScan DBSWIN	OPG
Akay et al.	2017	Turkey	16-71	211	Planmeca Romexis	CBCT
Roh et al.	2018	Korean	21-69	266	Adobe Photoshop 5	OPG
Hisham et al.	2019	Malaysia	16-69	300	Image J	OPG

The applicability of results from using the Kvaal et al. methodology on different populations reports a high degree of intra and inter observer correlation which indicates excellent agreement between observers and the reproducibility of the measurements. Therefore, the Kvaal et al. original methodology remains the same, but Kvaal et al. suggested dental ratios, age predictors, and age estimation equations were modified by some researchers, leading to mixed results (Table 4.6). Although the Kvaal et al. methodology was developed from six periapical radiographs, it has mostly been applied to digital OPGs because of the natural advantage of acquiring images of six teeth together in one radiograph.

Regarding the effect of sex on age estimation, no study has used sex as a predictor with the Kvaal et al. suggested predictors. Studies reported controversial age estimation results, using the Kvaal et al. methodology between males and females as few studies found difference between males and females. Whereas, some reported that no difference was found between them.

Table 2.6 SEE ± in years obtained from the Kvaal et al. method in different populations.

Study	Teeth (FDI) with SEE (years)								
	11/21 12/22 15/25 32/42 33/43 34/44	11/21 12/22 15/25	32/42 33/43 34/44	11/21	12/22	15/25	32/42	33/43	34/44
Kvaal et al.(1995)	8.6	8.9	9.4	9.5	10.0	11.0	11.5	11.5	10.5
Paewinsky et al.(2005)	5.6	-	-	-	6.4	-	-	-	-
Bosman et al.(2005)	9.5	9.2	9.9	9.7	9.8	9.3	11.6	8.2	8.1
Erbudak et al.(2012)	-	-	-	10.01	-	10.12	8.73	-	-
Talreja et al.(2012)									
(Group A)	12.08	12.08	12.40	12.79	13.25	11.87	13.30	13.89	13.28
(Group B)	11.90	11.27	12.46	11.17	13.40	12.62	13.43	-	12.75
Limdiwala et al.(2013)									
(Group A)	8.3	8.21	9.09						
(Group B)	9.45	9.5	9.88						
Misirlioglu et al.(2014)	-	-	5.88	-	-	-	7.39	7.89	7.54
Karkhanis et al.(2014)	8.36	8.99	9.60	9.36	9.64	9.52	10.22	10.90	10.53
Mittal et al.(2016)	7.97	8.59	7.51	8.15	8.53	7.89	8.85	7.95	7.58
Akay et al.(2017)	12.75	11.56	12.24	8.39	5.44	10.83	7.21	8.32	15.38
Roh et al.(2018)	10.7	10.4	12.6	11.9	12.3	13.1	14.1	14.0	14.2
Hisham et al.(2019)	12.01	11.07	12.72	10.46	11.19	11.42	12.01	12.91	11.58

2.2.1.2 Paewinsky et al. method

The method developed by Kvaal et al. was further elaborated and modified by Paewinsky et al. Pulp and tooth widths were measured at three root levels and converted into ratios as described by Kvaal et al. and used as predictors for age estimation (Paewinsky et al., 2005). Descriptions of the measurements are in (Table 2.7).

Table 2.7 Descriptions of the ratios and notation.

Width ratios at three tooth levels	Notation
Ratio between root and pulp width at enamel cementum junction (ECJ)	A
Ratio between root and pulp width at midway between levels A and C	B
Ratio between root and pulp width between apex and (ECJ) Level A	C

The measured values of Ratios A, B, C were compared in male and female teeth and no statistical difference was observed. Different correlation values were found between Ratios A, B, C with chronological age; therefore, three regression formulas were formed from each tooth using Ratios A, B, C as predictors for age estimation. Overall, the best correlations were found between Ratio A and chronological age in all teeth, apart from mandibular first premolars. Furthermore, the results indicated that Ratio A from the maxillary lateral incisor produced a high coefficient of determination (R^2=0.839) and less difference between estimated and chronological age (6.68 years) in all teeth. Overall, Ratios A, B and C of the maxillary lateral incisor produced the best results. Similarly, if the three ratios are combined, then the achieved results indicate higher correlation values, than using Ratios (A, B, and C) in isolation.

Overall, the obtained results of Paewinsky et al. are comparable to Kvaal's et al. original results. Additionally, the results indicate the applicability of the Kvaal et al. method on orthopantomograms (Paewinsky et al., 2005).

2.2.1.3 Age estimation using the Kvaal et al. and Paewinsky et al. formulas

Landa et al. compared the Kvaal et al. and Paewinsky et al. formulas using mandibular teeth from OPG to determine which formula is more accurate for age estimation (Landa et al., 2009).

The results suggest that estimated age from the Kvaal et al. and Paewinsky et al. formulas were both far from the chronological age and tended to overestimate the age. Conversely, Meinl et al.'s results suggest that estimated age was consistently underestimated in the Kvaal et al. formula and constantly overestimated by the Paewinsky et al. formula (Meinl et al., 2007). A possible explanation of the discrepancy between Landa et al. and Meinl et al. results obtained from the Kvaal et al. formula might be the selection of age range of the sample as Landa et al. selected 14-60 years old using OPG radiographs while Meinl et al. used OPG radiographs of 13-24 year olds. Due to far exceeding the estimated age over chronological age, these researchers do not support the applicability of these formulas for age estimation (Landa et al., 2009, Meinl et al., 2007).

2.2.1.4 Age estimation using the Kvaal et al., Paewinsky et al. and newly developed formulas

Erbudak et al. compared the accuracy of the Kvaal et al., Paewinsky et al. and newly developed formulas against chronological age (Erbudak et al., 2012). The correlation coefficient analysis between the Kvaal et al. suggested dental ratios and age showed that Ratios R, P, W, and L were significantly correlated with age; therefore, these ratios were selected as predictors in the Erbudak et al. formula for age estimation.

Furthermore, 12.17 to 27.63 years of difference was found using the regression formulas derived by Kvaal et al. and Paewinsky et al. and 8.73 to 10.12 years of difference was found from Erbudak et al. formula. The outcome of the study suggest that using the most correlated variables as predictors by Erbudak et al. formula produced lower standard error of estimation (SEE) as compared with the Kvaal et al. and Paewinsky et al. formulas.

Roh et al. compared the accuracy of the Kvaal et al., and Paewinsky et al. formulas, and their own developed formula. Roh et al. used the sum of the width ratios of A, B and C (Paewinsky et al. predictors) as a predictor for age estimation (Roh et al., 2018).

A difference of 12.61 to 18.84 years was found from Kvaal et al. proposed equation. Using the Paewinsky et al. proposed equation produced a difference of 15.14 to 30.55 years between estimated and chronological age. Generally, the Kvaal et al. formulas resulted in underestimated age, whereas the Paewinsky et al. formula resulted in overestimated age. This same tendency was observed by Meinl et al. (Meinl et al., 2007).

The Roh et al. developed formula found 10.4 to 14.2 years of differences between estimated and chronological age.

It can be concluded that using the sum of the width ratios from A, B, and C (the Roh et al. developed formula) produced better results than using Ratios A, B and C individually (Paewinsky et al.) and from using Ratios M and W-L (Kvaal et al.) as predictors for age estimation.

2.2.1.5 Finding suitable predictor from the Kvaal et al. and Paewinsky et al. predictors

Saxena et al. aimed to a find suitable predictor for age estimation from the Kvaal et al. and Paewinsky et al. suggested predictors (Saxena, 2011).

The correlation results suggest that Predictor C (pulp tooth width ratio at a mid-root level) is the most suitable predictor and significantly correlated with age in both sexes. This outcome is contrary to Paewinsky et al. who found that Level A (root and pulp width at the enamel cementum junction) was the most suitable predictor for age estimation (Paewinsky et al., 2005). This inconsistency may be due to a different selection of teeth as Saxena et al. selected a maxillary canine, whereas, Paewinsky et al. selected six teeth for the analysis.

2.2.1.6 A newly developed equation using the Kvaal et al. suggested predictors

Sharma et al. assessed the accuracy of their newly developed equation using the Kvaal et al. suggested predictors for age estimation (Sharma and Srivastava, 2010).

The results suggest that no significant difference ($p>0.05$) exists between estimated and chronological age in all teeth, except in mandibular and maxillary lateral incisors. Further results reveal that the strongest coefficient of determination was achieved from the mandibular first premolar (R^2=0.198) and the lowest coefficient (R^2=0.072) from a combination of three maxillary teeth. In contrast, Kvaal et al. results showed the highest values of the coefficient of determination were acquired from six teeth combined and lowest from the mandibular canine (Kvaal et al., 1995).

On the other hand, Hisham et al. found that the maxillary central incisor (SEE ± 10.46 years, R^2 =0.992) produced the best results and combined mandibular teeth (SEE ± 12.728, R^2= 0.035) produced the least accurate results using the Kvaal et al. suggested predictors in their age estimation equations (Hisham et al., 2019). Although a combination of teeth did not improve age estimation accuracy, the authors reaffirmed the reproducibility of the Kvaal method using OPG radiographs. Furthermore, Limdiwala et al. reported that 8.3 years of difference was found between estimated and chronological age from all six teeth using the Kvaal et al. suggested predictors in their age estimation equations (Limdiwala and Shah, 2013). Moreover, Mittal et al. reported that 7.51 years of difference was found between estimated and chronological age from three mandibular teeth using the Kvaal et al. suggested predictors in their generated age estimation equations (Mittal et al., 2016).

Bosman et al. used the Kvaal et al. original formula for age estimation and compared the accuracy of the obtained results with Kvaal et al. original results (Kvaal et al., 1995, Bosmans et al., 2005). The comparison of results reveal that no significant difference was found between estimated age and chronological age in all the Kvaal et al. suggested six teeth and three mandibular teeth:- whereas, a significant difference was evident in three maxillary teeth and all single teeth using the Kvaal et al. original formula. Moreover, 0.1 to 3.3 years of difference was identified between the obtained results using the Kvaal et al. original formula and the Kvaal et al. original results.

It can be concluded that the Kvaal et al. technique is applicable to OPG radiographs and that the results obtained from the Kvaal et al. original formula may lead to comparable age estimation results with chronological age. A possible explanation for this outcome might be population sample as both Kvaal et al. and Bosman et al. utilised the same small Caucasian population sample.

Conversely, a 5.88 to 7.88 years of difference was obtained between the estimated and chronological age using mandibular lateral incisors, canines, and premolars from the Kvaal et al. original formula. Although the difference between estimated and chronological age was slightly high when compared with the Bosman et al. results, it can be suggested that the Kvaal technique and formula can be applied to the OPG radiographs of mandibular teeth (Misirlioglu et al., 2014, Bosmans et al., 2005).

2.2.1.7 Age estimation using multiple predictors in a regression formula

Karkhani et al. compared the accuracy of individual and average values of the Kvaal et al. suggested predictors with Kvaal et al. original predictors (Karkhanis et al., 2014). The details of the different combination of predictors provided in (Table 2.8).

Table 2.8 Multiple predictors used in regression models of maxillary and mandibular teeth.

Teeth	Predictors combinations
Three maxillary teeth	Averaged M and W-L values (2 predictors) Individual M and W-L values (6 predictors)
Three Mandibular teeth	Averaged M and W-L values (2 predictors) Individual M and W-L values (6 predictors)
Six teeth	Averaged M and W-L values (2 predictors) Individual M and W-L values (12 predictors)

A difference of 9.367 to 10.534 years was found between estimated and chronological age using the Kvaal et al. suggested predictors in newly formed equations whereas, 7.963 to 9.608 years of difference were observed when using individual and averaged values of the Kvaal et al. suggested predictors.

Although no statistical test was performed to assess the comparison of results, it appears that including individual values of M and W-L and multiple predictors from a combination of teeth slightly improved the accuracy of the age estimation.

2.2.1.8 Comparison of estimated age by two different population formulas from two radiographic techniques

Talreja et al. divided the sample based upon paralleling (Group A) and bisecting techniques (Group B). A study compared the accuracy of the original Kvaal et al. formula and new population-specific formulas using principal component regression analysis applied to two radiographic techniques (Kanchan-Talreja et al., 2012).

A large variation in results was found from the Kvaal et al. original formula. A difference of 18.1 to 20.2 years was found between estimated and chronological age in Group A and 19.5 to 21.4 years of difference was found in Group B. Similarly, population-specific formulas found 11.87 to 13.30 years of difference between estimated and chronological age in Group A and 11.17 to 13.40 years of difference in Group B (Kanchan-Talreja et al., 2012).

It can be concluded that neither radiographic techniques nor population specific formulas improved the difference between estimated and chronological age.

2.2.1.9 Applicability of the Kvaal et al method using three–dimensional imaging

Recently, with the wide use of three–dimensional images in dentistry, Gulsan et al. evaluated the applicability of the Kvaal et al. method on CBCT and estimated age using the Kvaal et al. suggested predictors in newly generated age estimation equations (Gulsahi et al., 2018).

The best result was produced from the maxillary lateral incisor (5.44 years). Overall, the age estimated results from individual teeth produced better results than using a combination of teeth. The outcome of the study suggests that CBCT images and the Kvaal et al. methodology can be a useful tool for age estimation.

2.2.1.10 Summary of the Kvaal et al. method

The reasons for the variation in results of the Kvaal et al. method are multifactorial. The selection of age distribution, range and sample size distribution in the research could be the reason for different results. Another reason might be the obtained values of the predictors used in the studies, as different values from different studies were obtained.

The precision and accuracy of the Kvaal et al. method depends on the selection criteria of the radiographs. The main disadvantage of OPG radiographs is that the images do not produce fine details, unlike the periapical radiographs. If good quality and clear images of OPGs were used for analysis, then the estimated results might lead to acceptable results (Paewinsky et al., 2005, Bosmans et al., 2005). The quality of OPG depends also on the patient position relative to the X-ray tube when taking the radiograph. If the patient position is compromised, this will result in an unsharp and distorted image (Bosmans et al., 2005). Similarly, superimposition in the proximal surfaces of teeth, uneven magnification, and distortion of the image due to patient position and overlapping of structures, especially of the cervical spine in the incisor region, are problems associated with OPGs. These factors can influence image quality; thus, the obtained results might be compromised (Karkhanis et al., 2014).

Brightness, contrast, resolution, and magnification manipulations, and adjustments can also influence the reliability and accuracy of measurements. The reliability and accuracy of measurements influenced by these different image processing tools may affect the quality of the image, which may create differences in the results of digital measurements (Karkhanis et al., 2014).

Researchers have highlighted the problem in determining and defining reference points for measurements on radiographic images which might be another reason for the difference in results between the observers. The interpretation differences related to pulp between observers can be another reason behind the discrepancy of results. Reference points for pulp measurement could be difficult to determine on monitors because the border of pulp appears as a grey zone rather than a line. This appearance creates a problem for observers to select the inner, middle, or outer end of the zone (Paewinsky et al., 2005, Kolltveit et al., 1998).

Moreover, blurred edges of pulp could be another reason for different results between the inter-observer measurements. These blurred edges are produced because 3–D pulp is reproduced on a 2–D radiograph, thus, the edges of the pulp become blurred due to its cylindrical shape. These diffuse edges could cause the differences between the measurements of the tooth between observers (Kolltveit et al., 1998). To achieve the maximum accuracy of measurements, adequate training is essential for the identification of the landmarks (Paewinsky et al., 2005, Karkhanis et al., 2014).

Another possible explanation for the discrepancies in results is the lack of a use of a stereomicroscope for width measurements. This lack produces not only statistically different results, but also result in intra-observer variations (Willems et al., 2002).

2.2.1.11 Cameriere et al. Method

Cameriere et al. published a series of papers using PTAR as a predictor from 2–D radiographs for age estimation (Cameriere et al., 2006, Cameriere et al., 2004). In 2004, a new method was introduced for age estimation using a PTAR from maxillary canines from a digital OPG radiographs (Cameriere et al., 2004). Initially, ten and twenty points were used to measure the pulp and tooth areas, but this was modified with time (De Luca et al., 2011). At first, the method was designed for canines, but incisors and premolars were subsequently added (Cameriere et al., 2004, Cameriere et al., 2012, Cameriere et al., 2013). At the beginning Cameriere et al. used digital OPG radiographs, but, later a digitalised periapical was used as well to measure the pulp tooth areas. This method is more representative of age changes within the tooth than the linear measurements taken by Kvaal et al. Cunha et al. described the Cameriere et al. method as the best method for age estimation not because of the obtained results but due to the applicability and testing by many researchers on different populations. In addition, the Cameriere et al. method is practically suitable, quick, and cheap for estimating age (Cunha et al., 2009).

The literature reveals that studies related to the Cameriere et al. method can be divided into two parts:

- Work involving Cameriere et al. themselves
- Testing the Cameriere et al. method by different researchers

2.2.1.12 Work involving Cameriere et al. themselves

Cameriere et al. have published series of papers on a pulp tooth area ratio method for a age estimation using apposition of secondary dentine.

2.2.1.12.1 Finding a suitable predictor for age estimation

In a preliminary study, Cameriere et al. compared Kvaal et al. and Paewisnky et al. suggested age estimation predictors with a PTAR predictor to find a suitable predictor for age estimation (Cameriere et al., 2004, Kvaal et al., 1995, Paewinsky et al., 2005). Descriptions of the predictors are provided in (Table 2.9).

Table 2.9 Descriptions of the predicative variables.

Variables	Descriptions
p	Pulp/tooth length
r	Pulp/tooth length
a	Pulp/root width at mid-root level
b	Pulp/root width at midpoint level between ECJ level and mid-root level
c	Pulp/tooth width at mid-root level
AR	Pulp/tooth area ratio

Ten points for pulp and twenty points for teeth were used to measure the pulp tooth area of the right maxillary canine from OPG radiograph. The linear measurements were performed as described by the Kvaal et al., and Paewisnky et al. (Paewinsky et al., 2005, Kvaal et al., 1995). Later, all the measurements and area were converted into ratios to be used as predictors for age estimation.

The Pearson correlation coefficient results between all predictors and age indicate that AR best correlated (r=-0.92) with age, followed by the pulp/root width ratio (c) (r=-0.42) at the mid-root level. Thus, c and AR were used in the regression equation for age estimation. The output of regression analysis indicates strong correlation (R^2=0.84), with 5.35 years of difference between estimated and chronological age. An ANCOVA analysis found no significant difference between the mid-root level (c) and AR of males and females. However, only a small sample size was used to find suitable age estimation predictors but AR emerged as a new predictor of age estimation.

2.2.1.12.2 Testing pulp tooth area ratio as predictor for age estimation

Having determined that PTAR is better correlated than linear measurements ratios with age, Cameriere et al. applied their method to digital radiographs of maxillary teeth that belonged to Italian mummies to estimate the age at death (Cameriere et al., 2006).

Although the study utilised very small sample for age estimation, differences of -1.3 to + 8.6 years were found between estimated age and age at death. The findings suggest that PTAR as a single predictor can be used for age estimation (Cameriere et al., 2006).

2.2.1.12.3 Testing pulp tooth area ratio as a predictor from periapical radiographs for age estimation

Cameriere et al. then tested their methodology on periapical radiographs of maxillary and mandibular canines that belonged to an osteological collection of Caucasian origin (Cameriere et al., 2007b).

The results reveal that PTAR was significantly correlated with age. Further analysis results indicated that no significant difference (p=0.881) was found between the PTAR of males and females.

Furthermore, when the PTAR of maxillary and mandibular teeth were used together as a predictor of age then 4.06 years of difference was found between estimated age and chronological age which is better than using maxillary (5.44 years) and mandibular (4.46) PTAR separately.

The acquired results from periapical canine PTAR as a predictor are comparable to Cameriere et al. previous results using the PTAR of canine from OPG radiographs. Therefore, PTAR as a predictor obtained from periapical radiographs is applicable for age estimation. Furthermore, using PTAR as a predictor from combined teeth provided better results than using it as a predictor from individual teeth.

The authors concluded that, in the future, their methodology could be applied to a larger sample to reduce the difference between real age and estimated age at death. In addition, the authors recommended investigation into race and culture effects on age estimation.

2.2.1.12.4 Testing the accuracy of age estimation using a two radiograph techniques

Cameriere et al. compared the accuracy of pulp tooth area ratio obtained from two radiographic techniques for estimating age (Cameriere et al., 2007a).

Periapical radiographs of maxillary and mandibular canines of Caucasian origin were taken using labiolingual and mesial techniques from an anthropology collection, and the pulp tooth areas were measured as described by the Cameriere et al. method (Cameriere et al., 2004). Four predictors were developed and correlated with age. Details of the predictors are provided into Table (2.10).

Table 2.10 Four developed variables and their descriptions.

Variables	Descriptions
x_1	Maxillary canine pulp tooth area ratio acquired with labio lingual technique
x_2	Maxillary canine pulp tooth area ratio acquired with mesial technique
x_3	Mandibular canine pulp tooth area ratio acquired with labio lingual technique
x_4	Mandibular canine pulp tooth area ratio acquired with mesial technique

Pearson’s correlation results indicate that all the predictors were significantly correlated with age. Thus, all four variables were used in the regression models for age estimation. Details of the regression models are provided in (Table 2.11).

Table 2.11 Models and predictors descriptions.

Name of regression models	Description
Model 1	Combination of x_1, x_2, x_3, x_4
Model 2	Combination of x_1, x_2
Model 3	Combination of x_3, x_4

The output of the regression analysis from Model 1 was (R^2=0.94), which is slightly better than Models 2 and 3. In terms of age estimation, 3.62 years of difference was noticed between estimated and chronological age from Model 1, which again is better than Models 2 and 3.

The results indicate that the combination of predictors using different techniques provided better age estimation than single predictors with different techniques. In addition, the R^2 obtained from combining two predictors with two different techniques was better than that obtained from one technique.

The results obtained from two radiographic techniques were better than earlier studies of Cameriere et al. who used one radiographic technique to obtain the pulp tooth area (Cameriere et al., 2004). A possible explanation for this outcome is the usage of different techniques for capturing pulp tooth areas. However, other possible explanations could be small sample size and limited age range, as a slightly larger sample size was used in the two different radiographic studies.

2.2.1.12.5 Applying pulp tooth area ratio from periapical radiographs for age estimation

Age at death was estimated using PTAR as a predictor from maxillary and mandibular canines in 20th century male Prisoners in a Mexican sample with known age at death (De Luca et al., 2011).

Three proposed equations by Cameriere et al. were used to estimate age at death (Cameriere et al., 2007a). Although high R^2 was obtained, with 1.909 years of difference found between estimated age and real age at death, no information was provided regarding whether the results were obtained from maxillary, mandibular or a combination of both maxillary and mandibular PTAR.

Moreover, in another study, Cameriere et al. obtained a coefficient of determination (R^2= 0.971) using maxillary and mandibular PTAR as predictor. In addition, 2.89 and 3.17 years of standard error of estimation were found between estimated age and age at death using maxillary and mandibular PTAR. Furthermore, no significant difference was found between the PTAR of males and females (Cameriere et al., 2009).

Overall, the studies by Cameriere et al. strengthen the idea that PTAR from periapical radiographs reveal the technique's possibility as a predictor for age estimation. Further research is needed to examine more closely the links between PTAR, sex, and age in a large sample size (Cameriere et al., 2009).

2.2.1.12.6 Testing the accuracy and reliability of pulp tooth area ratio using inter–rater reliability

It is important to establish inter and intra rater reliability when conducting the measurements because these two–reliability tests are important aspects of measurement validity. Intra–rater reliability was performed to assess how consistent an observer is measuring the pulp tooth area, whereas inter–rater reliability refers to how consistent two observers are at measuring the same pulp tooth area (Underwood et al., 2017).

Cameriere et al. previous work demonstrated good intra–rater reliability but failed to show any inter–rater reliability. Therefore, a study was performed to test the inter–rater reliability of the measurements using Cameriere et al. work in blind trails (Azevedo et al., 2014).

For this purpose, Cameriere et al. suggested methodology was applied to obtain PTAR from a periapical maxillary canine from an osteological collection, and age was estimated using previous equations (Cameriere et al., 2007a).

The results of the Kolmogorov-Smirnov (K-S) test suggest normality existed between estimated and chronological age. The Bland-Altman plot indicates that no differences were found among the measurements of the observers. Similarly, around 4 years difference was found between the observer's estimated ages and the chronological age. Moreover, Intra class correlation (ICC) values were above 0.90 which indicates that the measurements were reliable among the observers. Similarly, p values were < 0.0001, which also indicates that measurements were highly significant among observers.

These results indicate high reproducibility, high correlations, and high agreement between observers of the applied method. The authors recommend that the Cameriere et al. method should be tested with different populations because it is user friendly, quick, and relatively economical.

2.2.1.12.7 Development of single regression model from two different sample

Over a period of time, the Cameriere et al. method improved in terms of its accuracy, reliability, and reproducibility between different samples. Therefore, an attempt was made to establish a common regression model for age estimation from two different populations (Cameriere et al., 2009). The constants and slopes of two regression analyses were compared. No significant difference between the intercepts or slopes were detected;-

thus, a common linear regression equation was established using maxillary and mandibular canines from Portuguese and Italian samples for age estimation.

The results of the maxillary equation found 4.24 years of difference between estimated and chronological age; whereas, 4.33 years of difference was found from the mandibular equation.

The authors recommend that age estimation can be achieved in different populations by using the common regression equation. To improve the difference between estimated and real age large sample size studies on different populations, races and cultures are also recommended.

Furthermore, estimated age from the Cameriere et al. and population specific formulas were compared with each other. Additionally, the estimated ages from both formulas were compared with real age to assess the accuracy of the formulas (Azevedo et al., 2015).

Individuals with all four healthy canines were included in the analysis. The comparability results indicate that significant differences were found when from the Cameriere et al. and Brazilian formula results were compared with each other. Similarly, a significant difference (p=0.001) was observed when estimated age from the Cameriere et al. and population specific formulas were compared with real age.

The results from the population specific formula were more precise than the Cameriere et al. formula for age estimation. Therefore, it might be useful to apply population specific formulas to achieve precise results. In this research, four teeth were selected per individual scan, which might be reason for the difference in results as bias was created.

2.2.1.12.8 Accuracy of pulp tooth area ratio in subjects over and under 65 age years old

The applicability of the Cameriere et al. method was tested on subjects aged both over and under 65 years of age in an Italian population (Cameriere and Ferrante, 2011). Digital periapical radiographs of canines were collected from a private dental clinic for people aged from 50 to 79 years old.

The sensitivity test results reveal that 85% of individuals aged 65 or above were correctly evaluated using maxillary PTAR and 88% were correctly evaluated using mandibular PTAR as a predictor for age estimation. Similarly, the specificity test result found that when using maxillary PTAR as an indicator 91% of estimated age was correct below 65 years of age; whereas, when using mandibular PTAR as an indicator, 89% of subjects were correctly estimated as being below 65 years of age.

Overall almost a 90% correct evaluation of age was achieved. These results reveal that pulp tooth area ratio is a reliable predictor for age estimation, even in old age.

2.2.1.12.9 Applying the Cameriere et al. method to lower premolars using orthopantomogram radiographs

In 2011, Cameriere et al. modified their methodology, replacing canines with mandibular premolars to estimate age (Cameriere et al., 2012). Different combinations of PTAR from single to mandibular premolars were used as predictors to assess the accuracy of the predictors for age estimation.

Digital OPG radiographs were selected from different radiological departments across Spain and pulp tooth area was calculated using the modified Cameriere et al. method. Additionally, age was estimated using PTAR in a new regression equation.

The results indicate that PTAR was significantly correlated with age; however, no significant difference was found between the PTAR of males and females; thus, sex was excluded from the regression models.

A difference of 7.35 to 7.99 was found between estimated and chronological ages using the PTAR of mandibular premolars teeth. In contrast, 5.31 to 6.38 years of difference was noted between estimated and chronological ages using different pairs of mandibular premolars. Regarding age estimation using different predictors, the PTAR obtained from four mandibular premolars produced better results than three, two and single PTAR of mandibular premolars. As four teeth together provided better results, this could be related to bias in the results.

2.2.1.12.10 Testing the Cameriere et al. method in central and lateral incisors

After analysing the canines and premolars, Cameriere et al. tried their methodology on central and lateral incisors (Cameriere et al., 2013). Periapical images of teeth were selected from an identified osteological collection and the described Cameriere et al. method and equations were applied for age estimation.

The results reveal that a significant difference was found between the PTAR of males and females. Further results reveal that the PTAR of upper lateral incisors produced 6.64 years of difference between estimated and chronological age. However, 10.90 years of difference was noted between estimated and chronological ages using the PTAR of lower lateral incisors. Regarding accuracy of age estimation, the difference of age estimation of central and lateral incisors was a little high when compared with canines. The authors believe that incisors are less reliable for estimating age than canines.

2.2.1.12.11 Evaluation of the Cameriere et al. method and anthropological methods

Cameriere et al. and different anthropological methods were applied to skeletons to evaluate the applicability of the methods. Furthermore, estimated age at death using the Cameriere et al. method was compared with other anthropological methods to assess whether it is in line with them (Cameriere et al., 2006, Fabbri et al., 2015a, De Luca et al., 2010). The names of the applied anthropological methods are in (Table 2.12).

Table 2.12 Details of the applied anthropological methods.

Skeletal element	Morphological feature	Reference	Year
Skull	Ectocranial suture closure	Meindl RS et al. Galera V et al.	1985 1998
Tooth	Dental wear	Miles AEW et al. Brothwell DR et al. Lovejoy CO et al. Gilmore and Grote	1963 1989 1985 2012
Fourth rib	Metamorphosis at the sternal rib end	Iscan MY et al.	1990
Ilium	Fusion of the iliac crest Metamorphosis of the auricular surface	Mayes et al. Lovejoy CO et al. Buckberry and Chamberlain	2003 1985
Public symphysis	Morphological changes of the articular surface	Todd TW. Brooks ST et al. Meindl et al. Suchey et al.	1921 1990 1985 1986
Humeral head	Fusion of the head	Mays et al.	2003
Ischiatic tuberosity	Fusion of the tuberosity	Mays et al.	2003
Sacrum	Fusion of vertebral body	Belcastro et al.	2008

Images of maxillary canines were obtained from skeletons for the implementation of the Cameriere et al. method, and age was estimated using PTAR as a predictor in regression analysis.

The results reveal that in 84% of cases, the estimated age using the Cameriere et al. method was in accordance with the anthropological method. Although a small sample was used in the study, the results indicate that the Cameriere et al. method results are comparable to other anthropological results (Cameriere et al., 2006).

On the other hand, Cameriere et al. used maxillary and mandibular canines and proposed equations to estimate the age (Cameriere et al., 2007a). The results indicate that the implementation of these methods on a sample vary from 11% to 85%. Dental wear was applicable in 85% of cases, sacral vertebrae in 20.4%, and pubic symphysis in 36.9%; however, the Cameriere et al. method was appropriate in 100% cases. The reason for the high implementation was the availability of canines that were still intact in the bone and well preserved. In addition, this result reflects the importance of teeth for age estimation. The comparability results of age estimation between the Cameriere et al. and anthropological methods found similarity in 89% of cases (De Luca et al., 2010).

Similarly, in another study Cameriere et al. results indicated that Cameriere method implemented in all the sample. On the other hand, the results varying from 72.2% to 88.9% by dental wear methods, 27.8% with cranial sutures, 44.4% by pubic symphysis, and 27.8% to 38.9% with the auricular surface. These results show that teeth remain preserved even when the skeleton is badly damaged. Age estimation using tooth wear methods excluded in the anthropological method as it is greatly influenced by life-style and diet. In 91.7% of results, the age estimated by Cameriere et al. remained in the range with results obtained using anthropological methods (Fabbri et al., 2015b).

Table 2.13 Studies reporting the use of the Cameriere et al. methodology.

Author	**Year**	**Population**	**Age Range**	**Total Sample**	**Teeth**	**Radiographs**
Cameriere et al.	2004	Italian	18-72	100	Maxillary canine	OPG
Cameriere et al.	2006	Italian	----	53	Maxillary canine	OPG
Cameriere et al.	2007	Italian	20-79	200	Maxillary canine Mandibular canine	Periapical
Cameriere et al.	2007	Italian	20-79	200	Maxillary canine Mandibular canine	Periapical
Cameriere et al.	2009	Italian Portuguese	20-84 20-84	200 258	Maxillary canine Mandibular canine	Periapical
Cameriere et al.	2011	Italian	50-79	90	Maxillary canine Mandibular canine	Periapical
Cameriere et al.	2012	Spanish	18-75	606	Mandibular premolars	OPG

Table 2.14 Summary of the Cameriere work with SEE ± years per tooth.

Author and Study Year	**Sample and Population**	**Age group**	**Software**	**SEE in Years and Teeth**
Cameriere et al. 2004	100 OPG Italian	18-72	AutoCAD2000	5.35 Maxillary Canine
Cameriere et al. 2006	43 Italian 10 Italian	21-81	AutoCAD2000	5.46 to 5.54 Maxillary Canine -1.3 to 8.6
Cameriere et al. 2007	200 Periapical Italian	20-79	AutoCAD2000	4.06 Maxillary and mandibular Canine 5.44 Maxillary canine 5.45 Mandibular canine
Cameriere et al. 2007	200 Periapical Italian	20-79	Adobe Photoshop	3.62 Maxillary and mandibular canine 4.74 Maxillary canine 4.47 Mandibular canine
Cameriere et al. 2009	258 Portugal 200 Italian 258 Portugal	20-84	Adobe Photoshop	2.89 Maxillary canine 3.17 Mandibular canine 4.24 Maxillary canine 4.33 Mandibular canine
Cameriere et al. 2012	606 OPG	18-75	Adobe Photoshop	5.75 6.38
Cameriere et al. 2011	103 Periapical Mexican	18-60	Adobe Photoshop CS4	1.909
Cameriere et al. 2013	427 Periapical Portugal	18-74	Adobe Photoshop CS4	7.03 Maxillary Central Incisor 6.64 Maxillary Lateral Incisor Mandibular Central incisor 10.80 Mandibular Lateral incisor 10.90

2.2.1.12.12 Summary of the outcomes of the Cameriere et al. method

The Cameriere et al. work assessed the relationship between PTAR and age using two dimensional radiographs. In addition, a newly developed method was established using PTAR as a predictor for age estimation.

- PTAR proved to be better than other predictors based on linear measurements for age estimation
- The Cameriere et al. method can be used on living and death peoples for age estimation
- Intra and inter rater reliability produced excellent results regarding the consistency of the method
- Estimated age using the Cameriere et al. method is comparable to and in-line with other anthropological methods
- Estimating age using periapical radiographs produced slightly better results than OPG radiographs
- Canines proved to be best teeth for age estimation
- A combination of maxillary and mandibular teeth produced better results than maxillary and mandible alone
- Age estimation using two techniques together produced better results than using a single technique.
- Controversial results were produced using the Cameriere et al. suggested formula for other populations.
- The majority of studies reported that no difference was found between male and female PTAR.

- Almost all the studies found a linear relationship between pulp tooth area ratio and age.

2.2.1.13 Testing of the Cameriere et al. method by different researchers

The Cameriere et al. method has been tested by different researchers estimating age in adults. Although the Literature does not provide a particular reason for using this method for age estimation in adults, it can be assumed that the applicability of the method for both living and deceased people could be the reason for its popularity. Additionally, Cameriere et al. achieved a high correlation between PTAR and age. Furthermore, the good consistency and reliability of the method might be another reason to apply and test the method in other populations. Another advantage of applying this method is that its applicability is not limited to one specific tooth, it can be used on other teeth as well to assess the PTAR relationship with age, sex and age estimation.

2.2.1.13.1 Age estimation using pulp tooth area ratio from periapical canine

Jeevan et al. found 4.28 years of difference between estimated and chronological age using the newly generated age estimation equation from maxillary canine PTAR (Jeevan et al., 2011). Moreover, 2.70 years of difference was observed when 16-44 years old pulp tooth area ratio of maxillary and mandibular teeth were used together. This age group was used because the majority of the sample was from this group. An unpaired t test suggested that no significant difference was found between male and female PTAR.

The outcome of the study suggests that maxillary canine PTAR produces better age estimation for the whole sample size but using a combination of maxillary and mandible canine PTAR produced better results in young and middle age. In addition, the study indicates that the selection of sample size and range is important for age estimation. These results are very similar to Cameriere et al. in which maxillary and mandibular canine produced better results (Cameriere et al., 2007b).

Moreover, Babshet et al. compared the accuracy of age estimation results from Cameriere et al. original formula and a population specific formula using periapical mandibular canine (Babshet et al., 2010).

The results suggest that Cameriere et al. original formula provided 11.01 years of difference between estimated and actual age in 55 .24% of the sample. By contrast, 4.38 years of difference was found between estimated and actual age using Cameriere et al. original formula on 90% of an Italian sample (Cameriere et al., 2007b). On the other hand, the matched control group results suggest that the Cameriere original formula provided 11.58 years of difference between estimated and actual age in 57.14% of the sample; whereas, the population specific formula yielded a difference of 10.76 years between estimated and actual age in 54.3% of the sample.

A scatter plot was used to assess the relationship between the PTAR obtained using the Cameriere et al. original formula and age. The plot illustrated that a regression model does not fit well with the trend of the data. Furthermore, the scatter plot between residuals and actual age found that there was over estimation of age among young age, and under estimation of age in old age.

With the forensic age prediction, the results were acceptable as they were close to the 10 years of difference between estimated and actual age (Solheim and Sundnes, 1980). These results indicate that the population specific formula produced marginally better results than the Cameriere et al. original formula. However, it may be the case that the population specific formula did not help much to reduce the difference between estimated and chronological ages.

2.2.1.13.2 Age estimation using pulp tooth area ratio from orthopantomogram canine

Dehghani et al. aimed to develop a population specific formula based on Cameriere et al. method to assess the chronological age using maxillary and mandibular canine PTAR from digital panoramic radiographs (Dehghani et al., 2018). The sample was divided into five age groups, from 16-64 years old, with intervals of ten years to determine in which age group the best results existed.

The results found no significant difference ($p > 0.05$) between the PTAR of males and females. Pearson's correlation indicates that PTAR of maxillary canine ($r=0.794$) produced a strong correlation with age compared with mandibular (0.282) and combined teeth ($r=0.685$) PTAR. Similarly, a scatter plot revealed that the PTAR of maxillary canines produced a very linear relationship with age compared with mandibular canine pulp tooth area ratio.

Regarding age estimation, over all maxillary canine PTAR produced the best results. Among the five age groups, maxillary canine provided the least difference between estimated and actual age in the age group of 25-34 years old.

The outcome of the study suggests that the PTAR of maxillary canine produced the best results and that the 25-34 years old age group had the best result age group results.

Similarly, Sakhdari et al. used the PTAR of maxillary canine from OPG radiographs to formulate an age estimation equation (Sakhdari et al., 2015). Regression analysis found 0.07 to 33.28 years of difference between estimated and chronological age in males.

Furthermore, no significant correlation (p=0.169) was found between male PTAR and age. Similarly, 0.36 to 17.17 years of difference was observed between estimated and chronological age in females. However, a significant correlation difference (0.0004) was found in the PTAR of females with age. These results are in accordance with a study by Torkian who exactly reported the same results (Arezou, 2015).

2.2.1.13.3 Age estimation using pulp tooth area ratio from periapical maxillary incisors

Zaher et al. assessed the reliability of age estimation using the PTAR of maxillary central and lateral incisors from periapical radiographs (Zaher et al., 2011). Linear analysis found that a weak coefficient of determination was achieved from maxillary central and lateral incisors with age. Despite these weak results, maxillary central incisors provided SEE ranging from 1.36 to 5.08 years, and maxillary lateral incisors provided 1.2 to 2.70 years. An ANCOVA analysis found no difference was found between the PTAR of maxillary central and lateral incisors between males and females.

2.2.1.13.4 Age estimation using pulp tooth area ratio from orthopantomogram of mandibular second premolars

Jeong et al. tested the applicability of the Cameriere et al. method on the lower second premolar using OPG radiographs (Lee et al., 2017a).

Based on the ANVOCA analysis, the PTAR of males and females showed a significant difference, indicating that sex is a definite contributor towards age estimation. The age estimation results revealed that using male PTAR as an indicator produced 11.1 years of difference between estimated and chronological age; whereas, 10.3 years of difference was found from using the PTAR of females. These results indicate smaller differences and more accurate results were achieved using female PTAR compared with males.

Moreover, Anastacio et al. validated the Cameriere et al. method by applying it to OPG radiographs of upper and lower second premolars (Anastácio et al., 2018). The results found no linear or non-linear relationship between the PTAR of premolars and age. Similarly, low values of coefficient were found with linear regression, which indicates low reliability of the PTAR as an age estimation predictor. Unfortunately, these results were not very encouraging, but this might be due to the small sample size.

2.2.1.13.5 Age estimation using pulp tooth area ratio from periapical three mandibular teeth

Babset et al. aimed to determine the age estimation accuracy using single and combinations of PTAR (Babshet et al., 2011) . Periapical mandibular lateral incisors, canines, and first premolars were used to measure the PTAR using the Cameriere et al. method.

Interestingly, intra observer results suggest a statistically significant difference in the measurements of lateral incisors and first premolars whereas, inter observer results indicate significant differences in the measurements of premolars. Regression analysis indicated that the PTAR of lateral incisors produced best results in a single tooth, while combining the PTAR of all teeth did not reveal any recognisable difference in results.

Overall, 12 years of difference between estimated and chronological age was found which falls outside the range proposed by Solheim and Sundnes (Solheim and Sundnes, 1980). The outcome of the study suggests that using a single tooth or a combination of teeth for age estimation did not much improve in the accuracy of age estimation.

2.2.1.14 Summary of studies using Cameriere et al. method

- A large variation of results was found between estimated and chronological age using the Cameriere et al. method.
- These variations could be due to ethnic factors in tooth morphology, secondary dentine deposition pattern and tooth abrasion related to eating habits.
- Another contributing factor to the differing range of results may be the use of both periapical and panoramic radiographs, as these two modalities are different regarding magnification, resolutions, and projection angle.
- Using multiple teeth PTAR did not improve the age estimation results. In contrast, Cameriere et al. used a combination of PTAR which produced a higher correlation with age and more accurate age estimation than single PTAR results.
- Comparison of age estimation between Cameriere et al. and other authors revealed a high difference between estimated and chronological age obtained from Cameriere et al. This discrepancy could be attributed to sample selection, as Cameriere et al. used a skeletal sample, which lacks soft tissues leading image clarity, whereas, living individuals possess soft tissue, which may cause the superimposition of images.
- Another reason for the discrepancy of results between Cameriere et al. and other results may be the selection of teeth. Cameriere et al. selected mostly canines whereas, other authors used maxillary incisors and other teeth more frequently.
- Controversial results were achieved using Cameriere et al. and population-based formulas for age estimation. Some authors recommend using a population-based formula while others disagree with this approach due to the large variations estimated and chronological age.

2.2.2 Age estimation using pulp tooth volume ratio from micro-computed tomography studies

μ-CT has been extensively used in dentistry to observe the structure of hard tissues, to measure the thickness of hard tissues, to understand root canal morphology, for craniofacial skeletal development and structure, tissue engineering, and for the mineral concentration of hard tissues. In short, μ-CT provides comprehensive and useful findings for research. On the other hand, it is not possible to use μ-CT every day in dental clinic because of the high radiation dose, small FOV, long scanning time for samples and the need for an extracted tooth for analysis.

In forensic dentistry, μ-CT is commonly used to collect information related to aging in bones and teeth. The collected information is very useful for the assessment of sex and age. Various researchers have investigated the relationship between the PTVR of different teeth and age using μ-CT and reported different results.

Vandevoort et al. used the PTVR of single teeth to explore the relationship of PTVR with age. Results from correlational analysis found a weak correlation (r=0.31) between PTVR and age (Vandevoort et al., 2004).

Someda et al. aimed to find a suitable region in teeth for age estimation (Someda et al., 2009). For this purpose, different regions of the pulp tooth volume of mandibular central incisors were measured and converted into ratios to correlate with age. Based on the measurements, the PTVR of five regions; - ratios of whole pulp and tooth, whole pulp and tooth excluding enamel, coronal pulp and crown, coronal pulp and crown excluding enamel and pulp canal and root were correlated with age.

The results showed that the obtained coefficient of determination from the ratios of the whole pulp and tooth, and the whole pulp and tooth excluding enamel were almost the same: however, the whole pulp and tooth excluding enamel (♂ 0.66, ♀ 0.78) produced marginally better results than the whole pulp and tooth (♂ 0.65, ♀ 0.77). Moreover, the PTVR of the crown (♂ 0.57, ♀ 0.69) produced the lowest coefficient of determination of all regions.

These coefficient of determination (♂ 0.67, ♀ 0.75) results are in accordance with those obtained by Agematsu et al (Agematsu et al., 2010). Although different methodologies were used to measure the PTVR, the obtained results were very similar. A possible explanation for these similar results by Someda et al. and Agematsu et al. can be attributed to mandibular central incisors as both studies used this tooth for analysis and it has the lowest morphological diversity in human permanent teeth (Someda et al., 2009, Agematsu et al., 2010).

Aboshi et al. obtained the ratios at four levels (crown region, coronal one third of the root, mid root and apical one third of the root) from μ–CT scans of lower first and second premolar (Aboshi et al., 2010).

A Pearson correlation between measured volume region and age found that coronal one third of the root correlated best and the region of the apical one-third of the root correlated least with age in both premolars. Using a combination of all four ratios did not find a better coefficient of determination than a single PTVR. However, the coefficients of determinations of all the PTVR were slightly better in lower second premolars than lower first premolars. Further analysis revealed a distinct reduction at coronal one third of the root level between both premolars in the 20 year old and 50 years old groups. Regarding age estimation, lower second premolars produced slightly better results than lower first premolars.

2.2.2.1 Pulp tooth volume ratio relationship with age

Regarding the relationship between PTVR and age, mixed results were observed. Vandevoort et al. and Somdea et al. attempted to fit linear relationships between PTVR and age (Someda et al., 2009, Vandevoort et al., 2004). However, Agematsu et al. suggest that 95% of PTVR displayed a density ellipse, with a less circular shape between males and females (Agematsu et al., 2010). Interestingly, Aboshi et al. found a sharp reduction of PTVR between the 20 year and 30 year age groups, and, a moderate reduction was noticed until the 50 year and 60 year age groups. However, after this stage, a sharp reduction was again noticed in the PTVR (Aboshi et al., 2010). Conversely, Sasaki et al. suggested that a non-linear relationship exists between PTVR and age. Finally, a sharp reduction was noticed in the PTVR from 15 to 30 years of age (Sasaki and Kondo, 2014).

Regarding difference in PTVR between sexes, mixed results were found. The majority of studies report that significant differences were found between the PTVR of males and females and that female are more strongly correlated with age than male PTVRs (Agematsu et al., 2010, Someda et al., 2009, Sasaki and Kondo, 2014). Conversely, Vandevoort et al. suggest that no difference was found between males and females PTVR (Vandevoort et al., 2004).

The difference between the results related to sex and relationship could be attributed to different methodologies used to measure PTVR, types of teeth selected for the analysis, the sample size, and the selection of age range. In addition, the studies that used small sample sizes reported a linear relationship; whereas, large sample size studies reported a non-linear relationship between PTVR and age.

2.2.3 Age estimation using pulp tooth volume ratio from cone beam computed tomography studies

In recent years, CBCT scans are a recently induced technology that is currently used in thousands of clinics. These CBCT scans are used when 2–D radiographs are not able to provide enough information for the correct diagnosis. Additionally, these CBCT scans allow forensic odonatologist to measure the pulp tooth volumes to estimate the age.

2.2.3.1 Age estimation using pulp tooth volume ratio from single rooted teeth

In 2006, Yang et al. attempted to assess the correlation between the PTVR of a small sample of single rooted teeth and age using CBCT images (Yang et al., 2006). The results found a coefficient of determination of 0.29 and 8.3 years of difference between estimated and chronological age. Although a small sample size was used for the research, the findings are encouraging for estimating age, but a large sample with equal age distribution is recommended for further analysis.

Star et al. found a moderate correlation (R^2=0.34) between the PTVR of single rooted teeth (Star et al., 2011). However, when single PTVR are considered against age, then incisors produced the best correlation (R^2=0.41) with age, while canines (R^2=0.07) appear to be the worst choice for age estimation. These findings suggest that the PTVR of single teeth is better to use, and that central incisors provide the best age estimation. These findings are consistent with Gulsahi et al., who reported the same observations about the PTVR of incisors and canines (Gulsahi et al., 2018).

Additionally, Biuki et al. results suggest that the PTVR of maxillary central incisors and canines had the strongest correlation with age. In general, Biuki et al. study had mixed results, and these are consistent with some studies but not with others (Biuki et al., 2017).

2.2.3.2 Comparison of predictive powers of pulp tooth volume ratio from different teeth

Haghanifar et al. aimed to evaluate the predictive powers of the PTVR of maxillary and mandibular central incisors and canines obtained from the sagittal and axial views of CBCT (Haghanifar et al., 2019). Regression analysis between the PTVR involving the sagittal and axial views against age indicate that maxillary central incisors (R^2= 0.586 and SEE =7.045) are more reliable, and that maxillary canine teeth (R^2= 0.586 and SEE =7.045) have the lowest predictor power for age estimation.

A comparison of the PTVR obtained from the sagittal and axial views indicates that the axial view (R^2= 0.48) has greater age predictor power than the sagittal view (R^2= 0.328) to predict age. These results are inconsistent with Lee et al., who reported that the sagittal view (R^2= 0.52) has stronger predictive power for age than the axial view (R^2= 0.42).

Similarly, a comparison of the PTVR of maxillary central incisors and canines indicate that maxillary central incisor possessed stronger correlation with age.

2.2.3.3 Age estimation using pulp tooth volume ratio from maxillary central incisor teeth

Aydin et al. used the PTVR of maxillary central incisors to assess the relationship between PTVR and age. A correlation analysis suggested a moderate correlation (r=0.615) between PTVR and age (Ugur Aydin and Bayrak, 2018).

Similarly, Porto et al. compared the PTVR of maxillary central incisors in five different age groups ranged from 22 to 70 years old with ten year of intervals and investigated the differences between the sexes. The results of the correlational analysis showed a weak correlation (R^2= 0.21) between PTVR and age. Further comparison between males and females indicated a significant difference between males and females and revealed a stronger association of females with age compared with men. Overall, a decrease in the PTVR was observed from the young age group to the old age group. A comparison of the PTVR of the 22-30 age group with the third, fourth and fifth age groups revealed a significant difference. Interestingly, no significant difference was found between the 51-60 and 61-70 age groups (Porto et al., 2015).

2.2.3.4 Age estimation by using two different voxel size

Voxel size is one of the important parameters of CBCT imaging. The size of the voxel is directly related to the quality of the image. In dentistry, 0.2mm, 0.3mm, and 0.4mm voxel size images are commonly available in the radiology archives. These sizes depend on the visualisation of the diagnostic task. The smaller the voxel size, the better the quality of the image. A study was performed using maxillary canine PTVR with 0.2mm and 0.4 mm voxel sizes to investigate the effect of voxel size resolution on age estimation.

A 0.236 coefficient of determination was achieved with both voxel sizes. Regarding sex, the results were 0.180 in females and 0.273 in males. Furthermore, using a 0.2mm voxel size provided a 0.285 coefficient of determination whereas, a 0.347 coefficient of determination was achieved with 0.4mm voxel size. A possible explanation of the high coefficient of determination from the 0.4mm voxel size is bias in the sample size, as, a 0.2mm voxel size contained 2.5 times more samples than the 0.4mm voxel size. Further analysis found no significant difference between the estimated and chronological ages between the sexes and voxel sizes (Adisen et al., 2018).

2.2.3.5 Age estimation from two different approaches

Asif et al. aimed to compare the strength of correlations obtained from two methods for age estimation (Asif et al., 2018). One method based on PTVR and other method based on pulp chamber and crown volume ratio. A CBCT image of maxillary central incisors with 0.3mm voxel size was used for the analysis.

Pearson correlation showed significant ($p<0.01$) negative relationship of both methods with age. In terms of strength of correlation, the pulp chamber crown volume ratio ($r=0.880$) showed stronger correlation with age as compared to PTVR ($r=0.799$). Furthermore, correlation values obtained from both methods show significant difference ($p=0.0049$). Pulp chamber and crown volume ratio showed superiority over PTVR method.

2.2.3.6 Age estimation using pulp tooth volume ratio from canines

The long survival rate and large pulp cavity of canine teeth make them highly popular for age estimation analysis. The pulp chamber volume and tooth volume were calculated from images of maxillary canines with a voxel size of 0.4mm. The result of the correlational analysis showed a moderate correlation ($R^2=0.389$) with age (De Angelis et al., 2015). On the other hand, the PTVR from the CBCT images of maxillary and mandibular teeth revealed a moderate correlation ($R^2= 0.38$) (Tardivo et al., 2011). Similarly, Tardivo et al. used CT scans from individuals with four canines and found that the PTVRs of maxillary canines possess the most powerful predictor for age estimation (Tardivo et al., 2014).

Age estimation using pulp tooth volume ratio from multiple detector computed tomography premolar

Sakuma et al. used the multiple detector computed tomography scans of mandibular first premolar images to assess age using PTVR. Their regression analysis suggests that a moderate relationship existed between PTVR and age (Sakuma et al., 2013).

2.2.3.7 Pulp tooth volume ratio, sexes and age estimation

Regarding PTVR, sex, and age estimation, some controversial results revealed by the studies. Mostly studies demonstrated that no significant difference found between PTVR of males and females (Yang et al., 2006, Gulsahi et al., 2018, Tardivo et al., 2014, Asif et al., 2018, Ugur Aydin and Bayrak, 2018). However, some results revealed that despite of no significant difference between males and females PTVR, but stronger association of age associated with females PTVR (Star et al., 2011, De Angelis et al., 2015, Tardivo et al., 2011). Additionally few studies found that males PTVR more closely associated with age than females (Biuki et al., 2017, Haghanifar et al., 2019, Asif et al., 2019, Sakuma et al., 2013).

There are limited numbers of 3–D studies based on teeth for age estimation available in literature. Additionally, studies with a large sample size and an even distribution of males and females are not available. It is important to include an equal number in each age group and an wide age range which covers the maximum population to avoid age mimicry. Therefore, extensive research needed to find the PTVR relationship with age based on even distribution of males and females.

Chapter 3. Age estimation using three–dimensional pulp volume

The pulp is housed in the centre of the tooth and is protected by the hard tissues that surround it. The tooth is the hardest tissue in the body, and its composition makes it highly resistant and minimally affected by physical and chemical factors over time (Someda et al., 2009). With ageing, secondary dentine deposition occupies the pulp space; thus, the pulp cavity narrows and the PV decreases proportionally with the deposition of secondary dentine (Gustafson, 1950, Bodecker, 1925). Therefore, researchers have used PV as a predictor to estimate age in adults (Ge et al., 2015, Ge et al., 2016, Andrade et al., 2019).

In 2004, Oi et al. observed age-related changes in the pulp cavity of extracted maxillary first premolar molars using µ-CT scans. The results reveal that PV decrease was not constant with age, and immense reduction was found in young to middle age compared with middle to old age (Oi et al., 2004).

Ge at al. provides the following reasons to use PV as an indicator for age estimation over PTVR (Ge et al., 2015, Ge et al., 2016).

- The decrease in the PV is directly associated with formation of the secondary dentine. Tooth volume is affected by the attrition of enamel; therefore, PTVR does not truly reflect the change from secondary dentine deposition.
- The PV provides better calculations than tooth volume due to the high image contrast between pulp and dentine.

Current literature suggests that using PV alone as a predictor is still in its infancy; therefore, it is worthwhile to conducting an analysis of PV against age, to understand the relationship between them. Furthermore, a difference in PV between the sexes has also been reported; - therefore undertaking a determination of sex as a predictor would additionally be valuable and has been included in this chapter to evaluate its effect on age estimation (Ge et al., 2015, Ge et al., 2016).

3.1 Aim

To investigate the relationship between human canines, sex and chronological age.

3.2 Objective

To assess the relationship between the PV of the left maxillary and mandibular canines singly and collectively with and without sex as a predictor against chronological age using CBCT images of Pakistani subjects aged 15-65 years old.

3.3 Research questions

3.3.1 Question 1

Is canine PV, reliable predictor for age estimation?

3.3.2 Question 2

Are there any differences between the PV of males and females for estimating age?

3.4 Methods

3.4.1 Ethical approval

Ethical approval was applied and obtained from the Advanced Digital Imaging Lahore (Letter No. 16062017/2). The ethical committee approved the application and a certificate was provided for data collection (Appendix 1). Since the data were acquired from Pakistan, no ethical approval was required from NHS Tayside Scotland (Appendix 2).

3.4.2 Sample size calculation

An a priori power analysis was conducted using G*Power. Assuming a small effect size of $f^2 = 0.02$, with an alpha level of 0.05, a target power of 95% and two predictors expected in the final model, the required sample size was found to be 776 (Erdfelder et al., 2009).

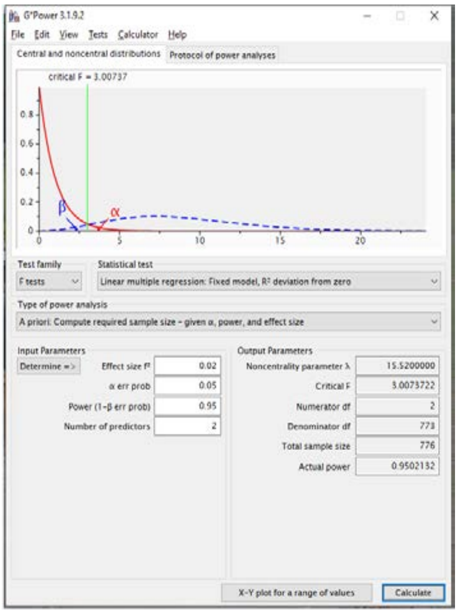


Figure 3.1 G* Power test for the calculation of the sample size.

3.4.3 Calibration of volumetric measurements

The accuracy of the volumetric measurements of teeth based on CBCT was evaluated. There is no difference between geometric shapes and volume error; therefore, a small block of gypsum was fabricated for the volumetric measurements (Park et al., 2017). The measurements were performed using a cuboid hand tool from the software Planmeca Rommexis® (Planmeca, 2017). After outlining the image, the software automatically computed the radiographic volume (Figure 3.3 A). The physical volume of the block was determined using the water displacement method. A 1000 ml graduated cylinder with 100 ml graduations was used to measure the volumes. The cylinder was filled with water up to the 300 ml mark (Figure 3.3 B). The block was immersed in the cylinder and the new level of water noted was 340ml (Figure 3.3 C). The final volume was obtained by subtracting the initial volume from the final volume, which was 340ml -300ml = 40 ml. The radiographic measurement of the block was 41.528. The physical volume of the block and the radiographic volume measurement were compared. A difference of 1.528 ml was observed between the physical and radiographic volumes.

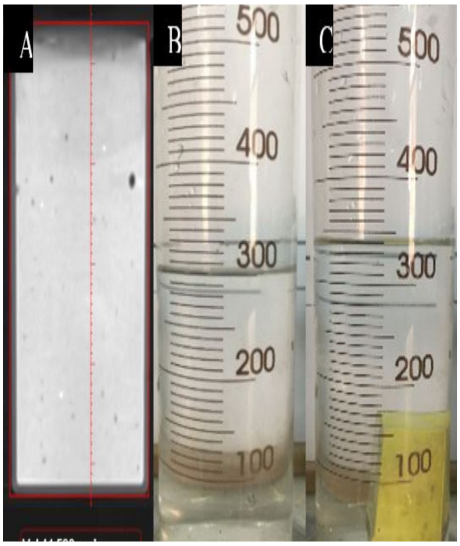


Figure 3.2 Volumes measured using different methods: (A) radiographic method (B) graduated measuring cylinder without sample (C) graduated measuring cylinder sample.

3.4.4 Correlation between maxillary and mandibular canine pulp volumes

To explore the most suitable tooth PV from which to gather PV data for the study, 192 PV of maxillary and mandibular teeth from 80 CBCT scans were tested. Later, a Pearson correlation was performed to determine the association between upper and lower and right and left maxillary and mandibular canine PV.

3.4.5 Test retest reliability

An intra class correlation coefficient (ICC 2-1, consistency) test was performed to measure the reliabilities of the measurements. A sample of 235 maxillary and mandibular teeth from the 80 CBCT scans was selected. The test-retest was performed between the first and second attempts, with a difference of three weeks.

3.4.6 Inter–rater reliability

The (ICC 2-1, consistency) test was carried out between two expert operators to assess the level of agreement between them. For the sample of 108 canines were analysed by the two raters from 30 scans.

3.4.7 Study design

This study is a retrospective cross-sectional design of the known age and sex of patients attending the Advanced Digital Imaging Lahore for dental diagnostics purposes.

3.4.8 Selection of the sample

A total of 717 (349 males and 368 females) CBCT images of left maxillary and mandibular canines were collected between December 2016 and September 2018 from the database of the Advance Digital Imaging Centre Lahore, Pakistan. The sample consisted of 258 maxillary and 313 mandibular canines from females, and 265 maxillary canines and 307 mandibular canines from males aged 15-65 years old. Each age interval contained a maximum of 8 males and females. The sample was balanced for age and sex and divided into 50 age intervals of 1 year each. All the images were anonymised with only age, sex, date of image recorded, and image scanned details provided.

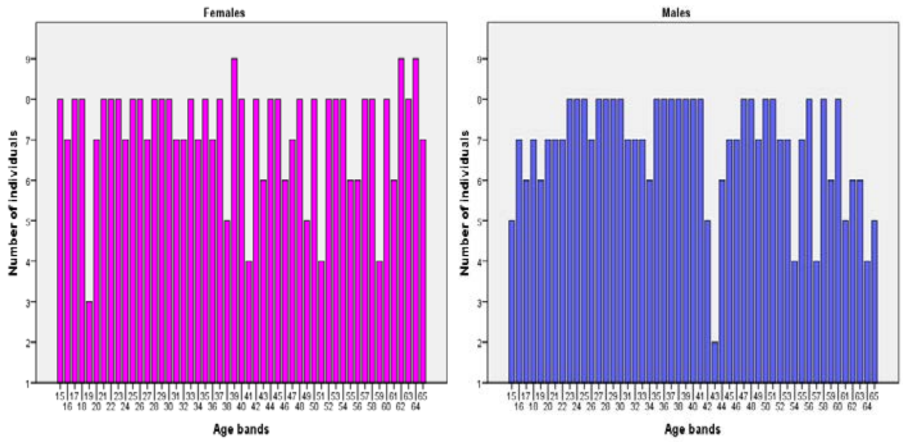


Figure 3.3 Distribution of sample size with age intervals and sex.

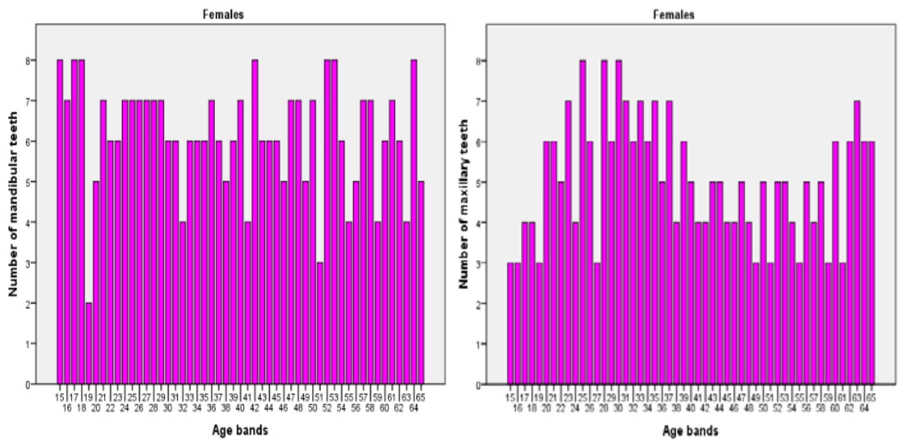


Figure 3.4 Distribution of maxillary and mandibular teeth by age in females.

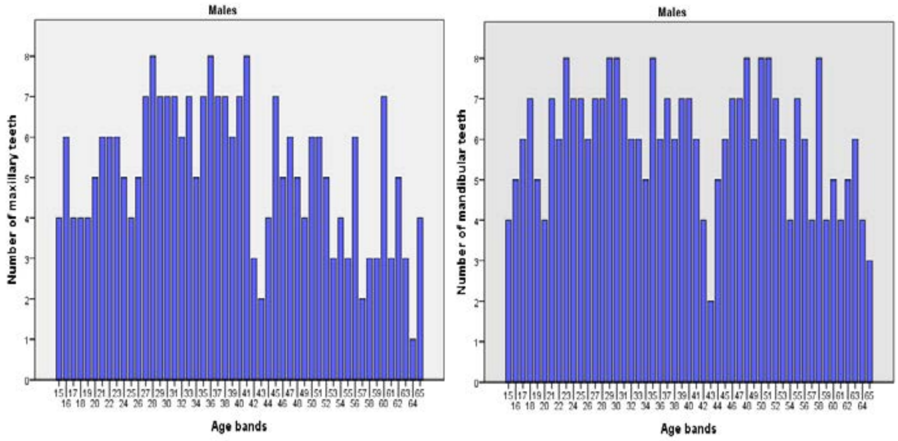


Figure 3.5 Distribution of maxillary and mandibular teeth by age in males.

3.4.9 Exclusion criteria for teeth

The exclusion criteria were as follows: pulp with caries, wear, restoration, impaction, artefacts, periapical lesions, root resorption, pulp with open apex, evident extensive wear and attrition, two canals, and pulp calcification.

3.4.10 Acquisition parameters

All the CBCT images were acquired from the Planmeca ProMax 3D Classic CBCT unit (Planmeca, Helsinki Finland) using 90 kVp tube current, 8 mA tube voltage, 796.6 DAP dose area (mGy × cm2) and 12.038 scanning time. The voxel size of images was 200 µm, field of view selection was Ø8.0 × 8.0 cm (401 × 401 × 401), and focal spot was 0.5mm.

3.4.11 Image reconstruction

The reconstruction process of the images mainly consisted of two stages: image acquisition and reconstruction. All images underwent numerous steps in these two stages to form the volumetric data. Initially, all the scans were exported to the Digital Imaging and Communication in medicine format (DICOM), a system of digital archiving that stores the images. The conversion of images into DICOM files (extension.dcm) was performed using the Planmeca Romexis® software. The software was downloaded from the Planmeca website (www.planmeca.com/software) for the volumetric analysis.

3.4.12 Transfer of the DICOM files

First, the DICOM files were transferred to the software by double clicking the Romexis icon  on the desktop. The images were added by clicking the ‘add images’  icon. Once the selected files loaded, then the ‘start viewer’ icon  double clicked  to begin the volumetric analysis.

Figure 3.6 Image transfer into the Planmeca Romexis viewer.

3.4.13 Opening a three–dimensional volume

The 3–D module of the software was utilised for the volumetric analysis. Once the 3–D module is opened, it consists of four tabs: volumes, explorer, panoramic and implants.

3.4.14 Explorer tab

In the next step, the explorer tab  was double clicked  . This tab contains the image information. The explorer tab opened the DICOM file. The 3D volumes were displayed simultaneously in three (coronal, sagittal, and axial) multiplanar reconstruction (MPR) views. In addition, a 3–D rendered view was also displayed. The reconstruction views consisted of orthogonal plane indicators. The orientation lines in each view have a relation between the orthogonal planes. The orientation lines were red for sagittal view, green for the coronal view and blue for the axial view. All the views were connected to each other, meaning that moving the mouse  in one view affects the other views.

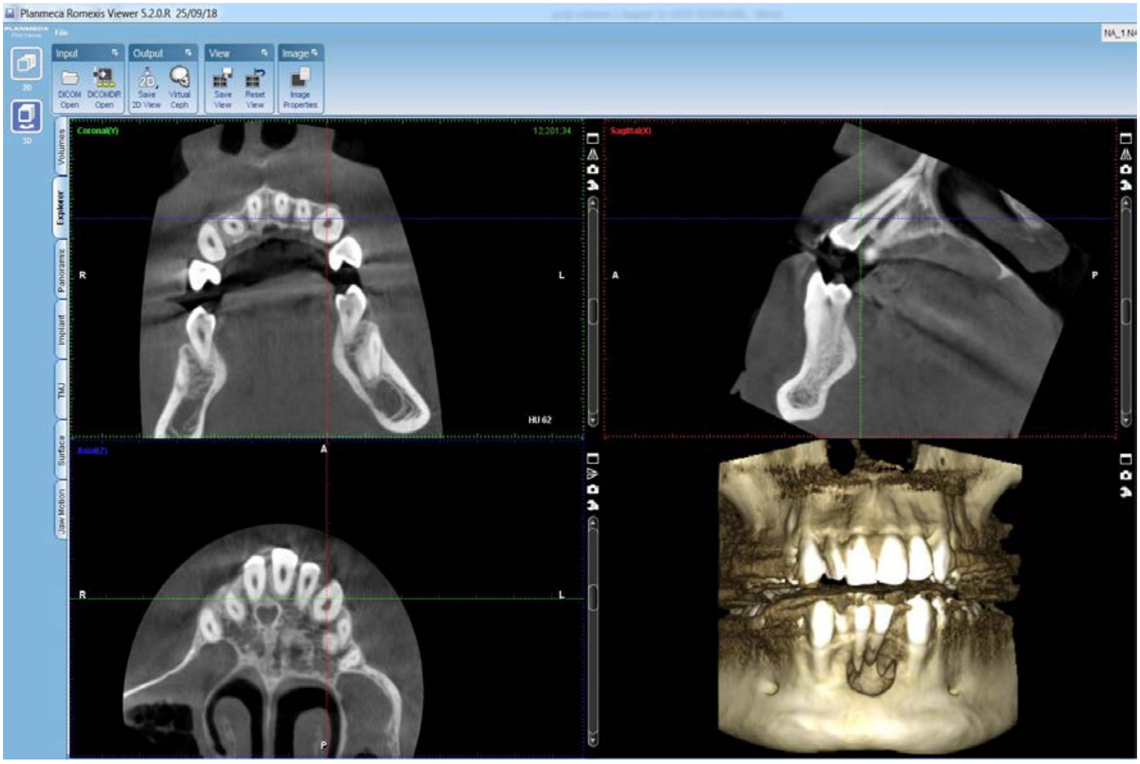


Figure 3.7 Three multiplanar reconstruction views with Three–dimensional rendering.

3.4.15 Volume tab

The volume tab was double clicked to open the 3–D volume. This tab provides information about the exposure parameters; for example, exposure date, image size, voxel size (µm), serial number, tube current, tube voltage, scanning time, and dose area product. In addition, the axial-view is displayed in thumbnail at the right side of the scan.

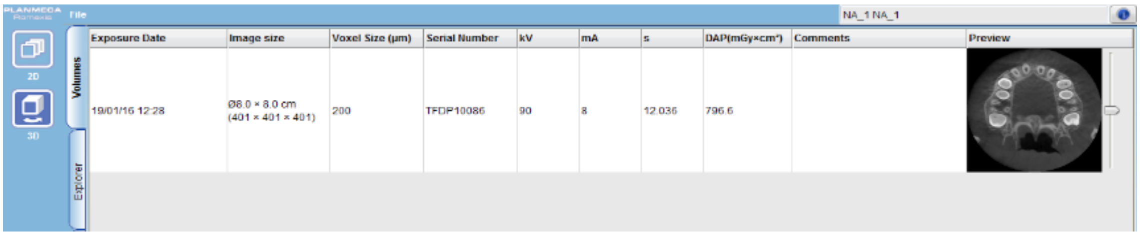


Figure 3.8 Details of exposure parameters.

3.4.16 Volume navigation

First, the image resetting was performed by clicking the reset orientation button. The canines were selected in the all view, one by one, using the mouse. Three–dimensional volume navigation was performed with the left and right mouse button. The left mouse button was used to move the volume and the right mouse button was used to rotate the volume. While moving and rotating the volume the orthogonal planes remained at right angles. The volume navigation moved in all planes throughout the tooth to assess any abnormality.

3.4.17 Plane navigation

The plane navigation was used to coincide all of the orthogonal planes. The volume remained static while the orthogonal planes were moved and rotated inside the volume. The sagittal plane was selected for the PV measurements. Similarly, volume navigation and plane navigation were also performed to assess the abnormality.

3.4.18 Volume orientation indicators

For the orientation of the image, the anatomies are indicated with anterior (A), posterior (P), left (L) and right (R) side on the corner views.

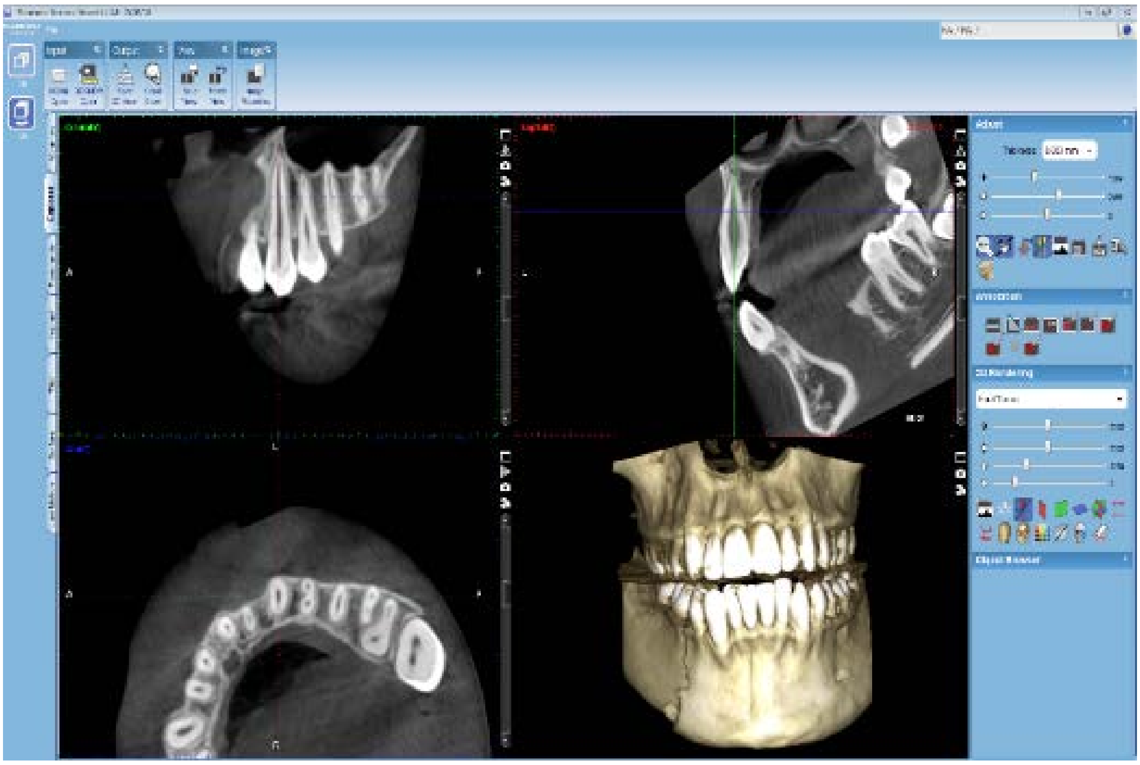
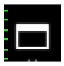
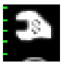
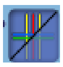


Figure 3.9 Volume and plane navigation in orthogonal planes.

3.4.19 Adjusting volumes

The selected views were maximised using the maximise button  located at the top right corner of each view. The wrench shaped button  on the top right of the view was used for the number of images, thickness, and slice interval. The show/hide orientation button  was clicked to hide the orientation lines (green, red, and blue) to facilitate a comprehensive view for the volume measurements.

3.4.20 Slice thickness and interval

The slice thickness and interval values are usually established by the investigator in accordance with the diagnostic task. It is recommended that, for minor and minute visualisations of structures, the slice thickness and interval should not be increased (Pauwels et al., 2015a). Image quality is impacted by the image noise, which is related to the slice thickness. It is important to keep a balance between slice thickness, image noise and the diagnostic task. Using a thinner slice increases the image noise but improves the diagnostic information about the small structure. On the other hand, studies indicate that slice thickness and interval up to 1mm can be chosen for volume calculations on CBCT images (Sezgin et al., 2013, Chadwick and Lam, 2010). Therefore, the pulp slice thickness and interval paired with the scan voxel size is 00.2 mm.

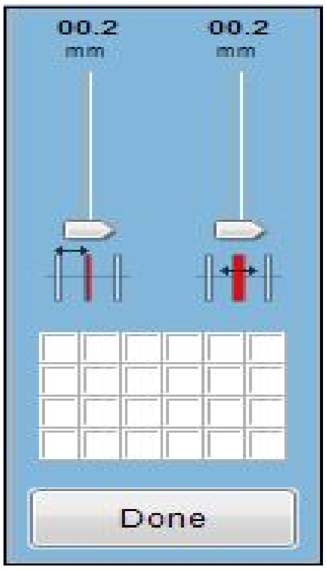


Figure 3.10 View-port settings for slice thickness and intervals. Left one is for interval and right one is for thickness.

3.4.21 Number of slices

For pulp measurements, pulp was divided into 18 slices. During the analysis pulp appeared between 4 and 14 slices. The initial and last slices were kept empty so that the pulp is always contained within the 18 slices.

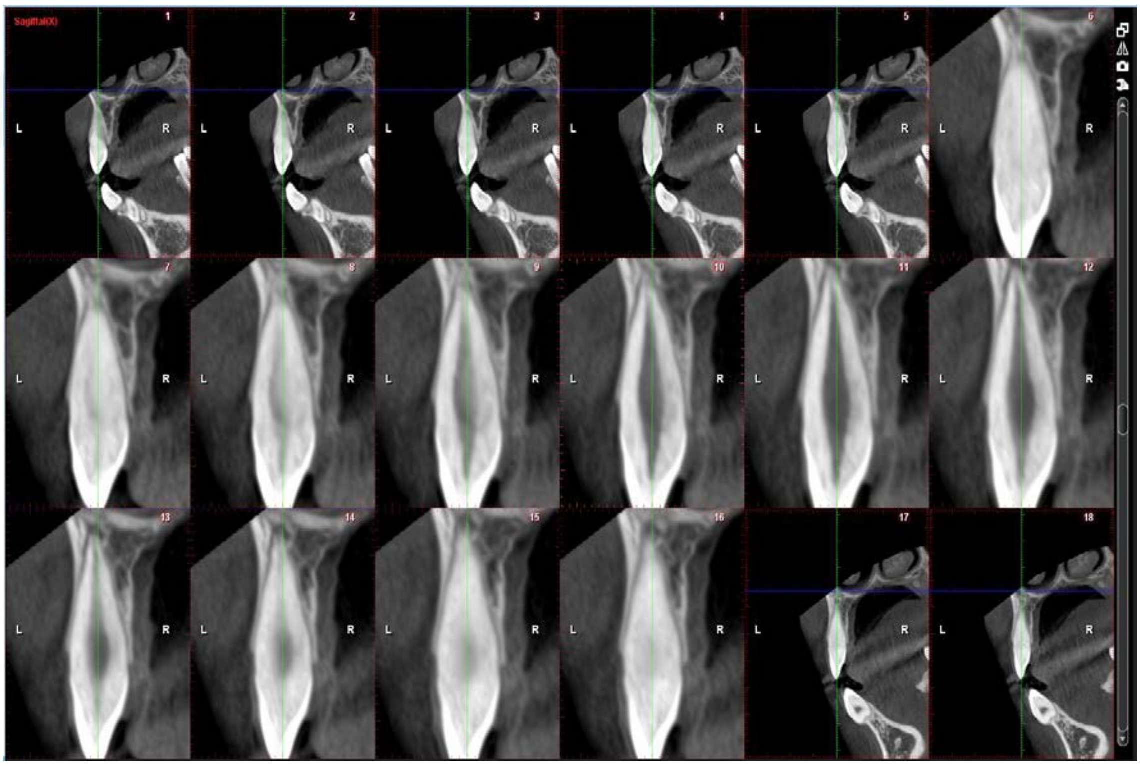
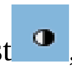
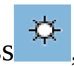
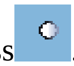
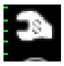


Figure 3.11 Sagittal view of the left maxillary canine.

3.4.22 Contrast, brightness, sharpness and toggle zoom

A range of 850–1030 was selected for contrast , 1820–2110 for brightness , and 3–10 for sharpness . Each image was maximised  for improved viewing and measurements.

3.4.23 Pulp outline tracing

The ‘free region grow’ icon  was selected from the annotation tools for pulp tracing. The outline of the pulp was drawn manually using a minimum of 10 points. The dot placement begum from the mesial end of the root, followed the outline, and ended at the distal end of the root. The wrench shaped button  was used to hide the orientation lines for better viewing and measurements.

3.4.24 Calculated Three–dimensional pulp volume

Following placement of dots around the outline of the pulp, the create region icon 

was clicked to display the PV. The calculated PV displayed along the pulp tracing in the selected slices.

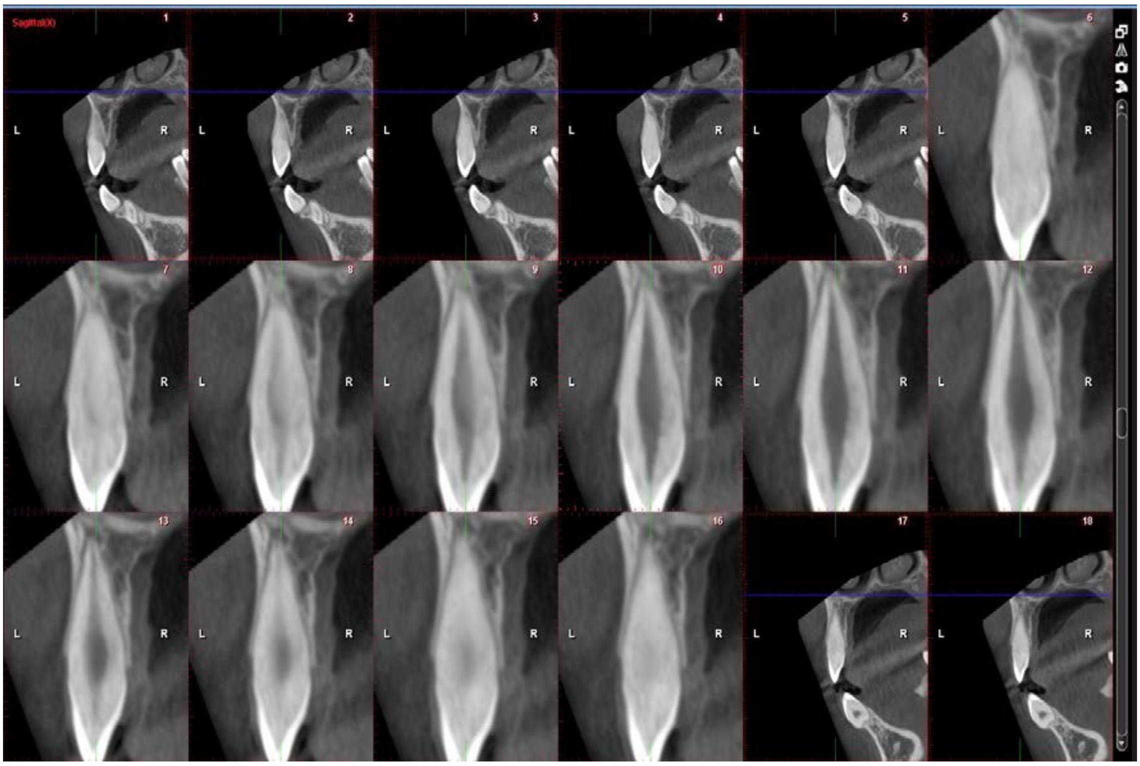


Figure 3.12 Removal of orientation lines for better viewing and measurements.

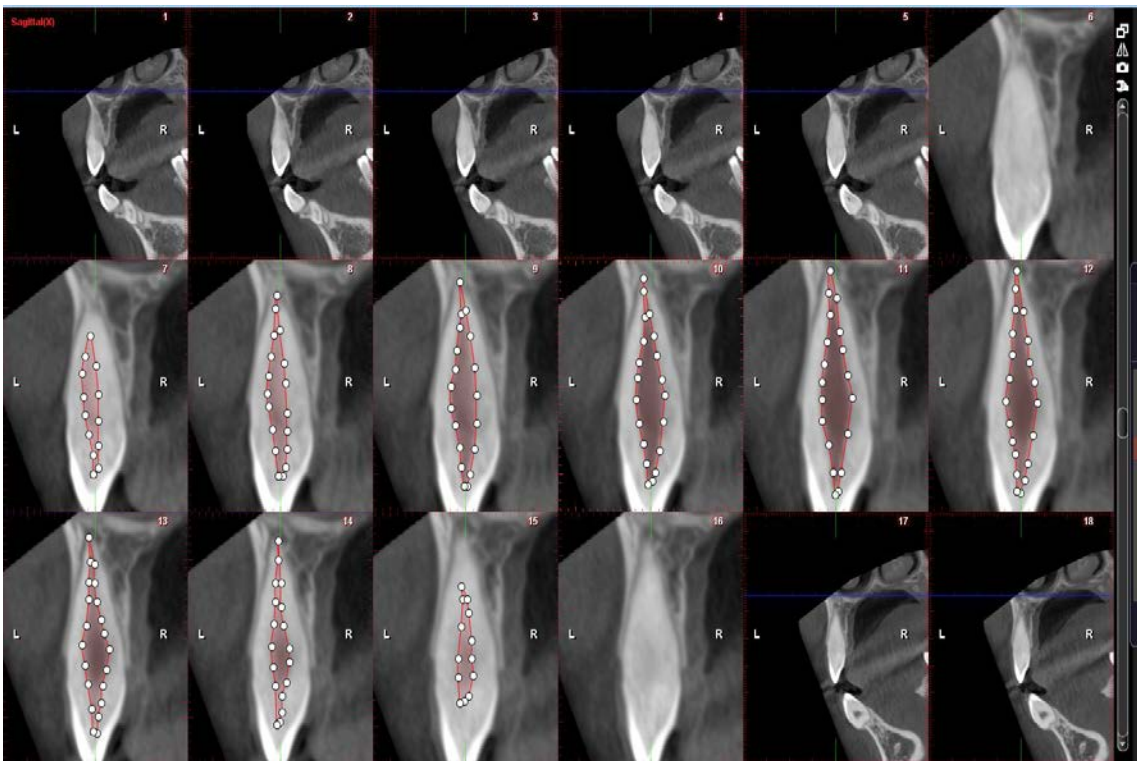


Figure 3.13 Tracing of the pulp outline.

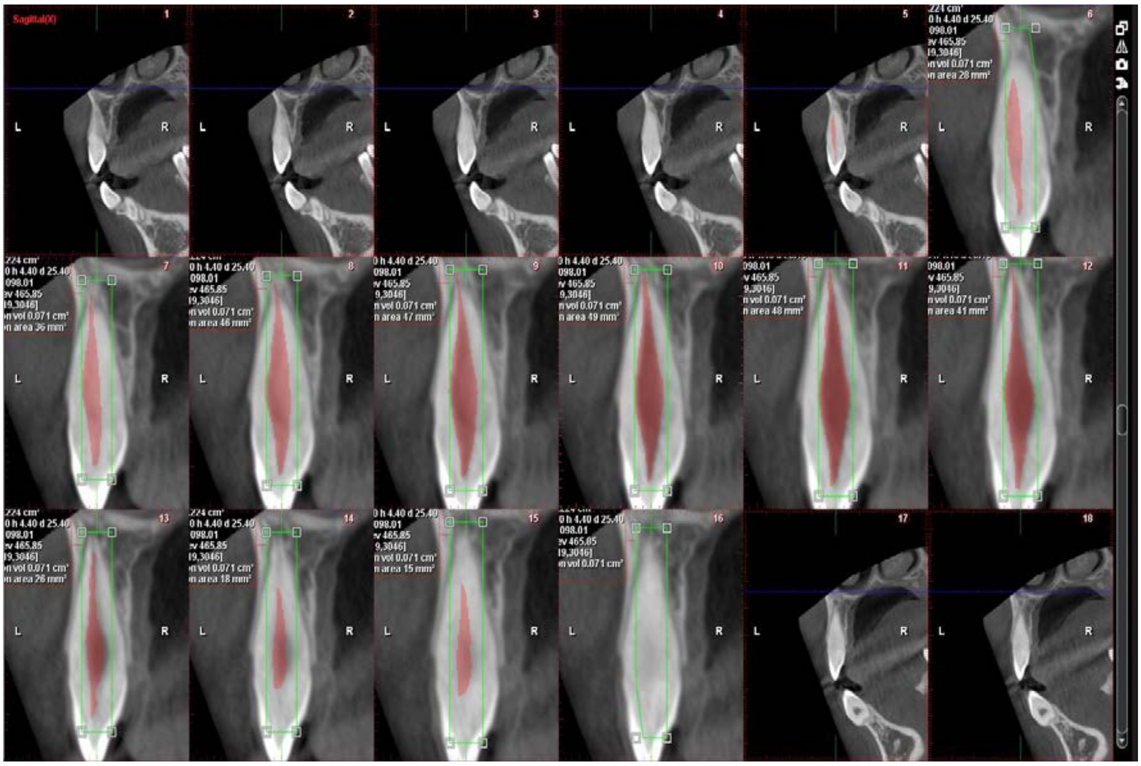


Figure 3.14 Obtained three–dimensional pulp volume.

3.5 Results

3.5.1 Correlation between maxillary and mandibular canines

A Pearson correlation test was conducted to evaluate the relationship between the maxillary and mandibular PV. Statistical analysis (ICC 2-1, consistency) test revealed that the comparison between the PV was not significant ($P > 0.05$). Hence, no difference was found between the maxillary left and right, the mandibular left and right, the maxillary left and mandibular left and the maxillary right and mandibular right canine PV. Therefore, the left maxillary and mandibular canine were selected for the analysis. The details and results of the comparison are provided in (Table 3.1) and (Appendix 3). The results of the Pearson's correlation ranged between 0.844 and 0.957 which suggests high levels of correspondence.

Table 3.1 r values by sex between left, right, upper, lower maxillary and mandibular canines.

Comparison between PV	**N teeth ♂**	**r**	**N teeth ♀**	**r**
Maxillary left and right	20	0.943	24	0.914
Mandibular left and right	33	0.957	26	0.944
Maxillary left and mandibular left	22	0.925	21	0.844
Maxillary right and mandibular left	21	0.941	25	0.845
Total	96		96	

3.5.2 Intra class correlation results

An intra-class correlation coefficient (ICC 2, 1- consistency) was performed to assess the test–retest and inter–rater reliability.

3.5.3 Test–retest reliability

The consistency of the measurements and the reliability of the measurements over time were assessed using test–retest reliability. The results ranged between 0.933 and 0.970 (Table 3.2). The reliability coefficients values were ≥ 0.9, which indicate a high correlation (good reliability) (Appendix 4).

Table 3.2 Correlation Coefficient values by sex undertaken by the first observer.

Comparison between PV	N teeth ♂	Correlation Coefficient	N teeth ♀	Correlation Coefficient
Maxillary left	25	0.967	27	0.970
Maxillary right	23	0.951	30	0.933
Mandibular left	35	0.951	30	0.965
Mandibular right	34	0.946	31	0.968
Total	117		118	

3.5.4 Inter–rater reliability

A level of agreement between the raters was assessed using inter–rater reliability. The obtained values ranged between 0.922 and 0.981. Thus, the raters agreement was above ≥ 0.9 which indicate high agreement and acceptable results (Table 3.3) (Appendix 5).

Table 3.3 r values by sex between left, right, upper, lower maxillary and mandibular canines undertaken by the first and second observers.

Comparison between PV	N teeth ♂ ♀	Correlation Coefficient
Maxillary left	23	0.981
Maxillary right	25	0.980
Mandibular left	31	0.937
Mandibular right	29	0.922
Total	108	

3.5.5 Inspection of normality

To assess the normality of the distribution, a histogram was constructed to evaluate the shape and spread of the data. The empirical distribution of the data was approximately bell shaped which resembles normal distribution. Due to the large sample size, the slight lack of normality was not overly concerning.

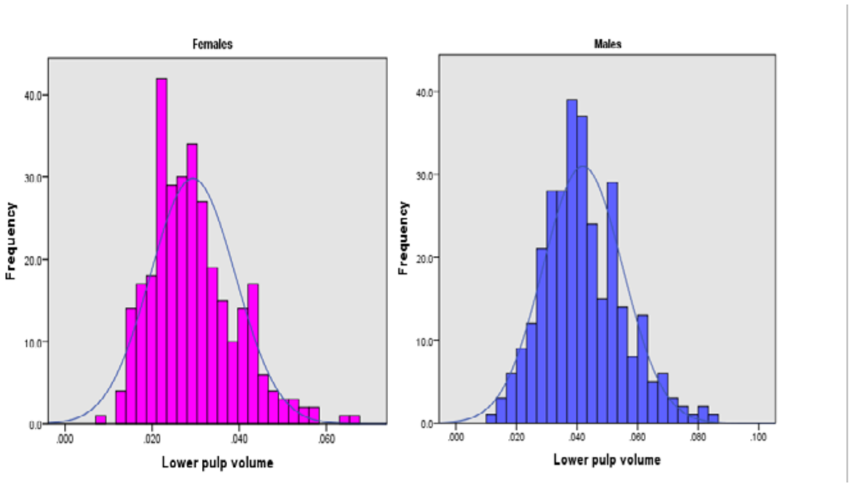


Figure 3.15 Histograms showing uniform pattern of mandibular pulp volumes.

3.5.6 Descriptive statistics results

A brief summary of the measured data is shown in (Table 3.4). Although no statistical tests were performed to detect the differences, there was a noticeable difference in the mean values between males and females and the confidence intervals do not overlap.

Table 3.4 Descriptive statistics of left maxillary and mandibular canine by sex. PV= pulp value. CI= confidence interval. SD=standard deviation. IQR= Interquartile range.

Gender	**Scans**	**PV**	**N**	**Mean**	**95 % CI for mean**		**S. D**	**S. D**		**Range**	**IQR**
					lower	**upper**		**min**	**max**		
Females	368	UPV	258	0.030	0.029	0.031	0.009	0.011	0.070	0.059	0.012
		LPV	313	0.029	0.028	0.030	0.009	0.009	0.064	0.055	0.012
Males	349	UPV	263	0.044	0.042	0.046	0.014	0.011	0.090	0.079	0.017
		LPV	307	0.042	0.040	0.044	0.012	0.016	0.082	0.066	0.016
Total	717		1141								

3.5.7 Formation of Regression Models

All the calculated PV were tabulated into an Excel sheet and compared with age and file numbers. An R statistical program and SPSS were used for further investigation. Six regression Models with variables such as PV with and without sex were formed. The details of the Models are in (Table 3.5).

Table 3.5 Six Models and predictors.

Models	**Predictors**
Model 1	Left maxillary PV
Model 2	Left mandibular PV
Model 3	Left maxillary PV and sex
Model 4	Left mandibular PV and sex
Model 5	Left maxillary and mandibular PV
Model 6	Left maxillary and mandibular PV and sex

3.5.8 Linear regression analysis

A linear regression analysis was carried out to determine the outcome of the PV, sex, and age. A scatter plot was used for each Model to visualise the correlation between the variables. Each of the six Models was used one by one, so that the best correlated Model could be selected for further analysis. The strength and association between the variables were described through the coefficient determination. Regression analysis revealed that all Models significantly correlated with age (Table 3.6). However, Model 4 displayed the highest predictive power; therefore, Model 4 was selected for further analysis.

Table 3.6 Regression values of the six Models.

Models	**Predictors**	**R^2**
Model 1	Left maxillary PV	0.26
Model 2	Left mandibular PV	0.26
Model 3	Left maxillary PV and sex	0.31
Model 4	Left mandibular PV and sex	0.33
Model 5	Left maxillary and mandibular PV	0.22
Model 6	Left maxillary and mandibular PV and sex	0.29

3.5.9 Diagnostic test and checking assumptions for Model 4

Some statistical diagnostic tests were carried out to check the properties of the Model and some assumptions were made, to achieve valid and reliable results. First, the studentised residuals were calculated between the observed and expected values. A graph was plotted between the residuals and fitted values (Figure 3.16). The graph revealed evidence of non-linearity. Several transformations (square root, log, reciprocal) were attempted to correct the non-linearity, but none were found to improve it.

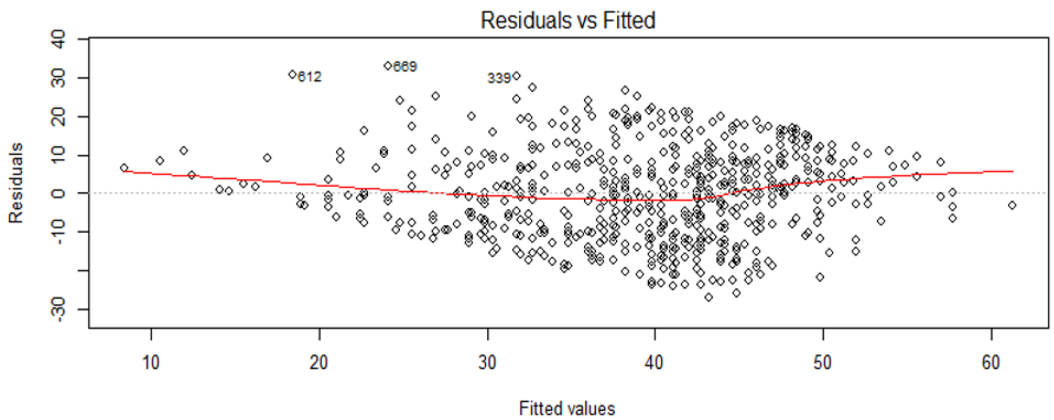


Figure 3.16 Plots of residuals against fitted values.

Model 4 was checked for the extreme studentised residual scores. There was no value exceeding ± 3, which means that there were not extreme scores. Furthermore, Cooks distance was used to find the influential outliers in the predictors. No values of ≥ 0.031 were found, indicating that no individual scores negatively affected the Model and biased the results.

A Durbin Watson test was conducted to measure the autocorrelation in the residuals. The test found a significant p value, which indicates a serious problem with non-independence. The errors in Model 4 are not random: when Model 4 predicts someone very young, they are almost always older; conversely, when it predicts someone very old, they are almost always younger. The issue is linked to the non-linearity mentioned previously.

Finally, multicollinearity was tested to assess whether the variables highly correlated in Model 4. The results revealed that there was not problem with multicollinearity.

3.5.10 Poly nominal regression analysis of Model 4

The data has a slight nonlinear variation in the start and in the end. Since the straight line in the linear regression unable to capture the pattern of the data therefore to overcome the under and over fitting in the data poly nominal regression was carried out for Model 4. In order to generate Poly nominal regression equation, powers such as PV^2, PV^3 and PV^4 were added to the linear regression equation.

A poly nominal regressions (PV^2, PV^3 and PV^4) along with quadratic and cubic were tried. The quadratic and cubic models showed statistically significant improvement over the standard linear model. However it did still suffer from autocorrelation/non-independence, just like linear Model 4.

A cubic polynomial with the curve provided the better fit than the linear line. The cubic line looked best when fitted to the graphs, however when tried adding a cubic polynomial, it was not a statistically significant improvement over the quadratic polynomial. Additionally, the R^2 of linear model of Model 4 was 0.33 which also slightly increased to 0.36 in poly nominal regression.

3.5.11 Descriptive statistics

The counts, means, and SD of PV of Model 4 are reported for different age bands below.

Table 3.7 Descriptive statistics of Model 4 with age and sex.

					Counts					
	15-19 group	**20-24 group**	**25-29 group**	**30-34 group**	**35-39 group**	**40-44 group**	**45-49 group**	**50-54 group**	**55-59 group**	**60-65 group**
Females	33	31	35	28	31	31	30	32	27	35
Males	27	32	35	32	34	24	34	33	29	27
					Standard Deviation					
Females	0.011	0.009	0.007	0.008	0.009	0.007	0.007	0.006	0.008	0.007
Males	0.014	0.011	0.010	0.010	0.009	0.010	0.011	0.010	0.011	0.008
					Means					
Females	0.043	0.035	0.030	0.031	0.030	0.028	0.025	0.024	0.023	0.023
Males	0.058	0.050	0.044	0.047	0.042	0.042	0.040	0.034	0.033	0.029

The relationship between PV and chronological age is expressed in (Figure 3.17). As previously indicated, there is a clear non-linear relationship that highlights the problems associated with trying to fit a linear model to this data. An independent t–test revealed that the difference in PV between males and females was statistically significant (p=0.000). A non-linear relationship between PV and age was found. This non-linear relationship appears to be the result of a variable rate of change for PV throughout life. The rate decreases more rapidly in early life, levels off in middle age, and resumes a more rapid descent in old age. A novel finding, however, is that the relationship is not a simple linear one, nor an easy one on which to model an exponential relationship, as reported by others; it is an odd S-shaped function that is rather more difficult to model. The detection of this function by the authors and not others is probably due to the large homogenous sample across all age used in this present study.

This results of this present study reveals a significant difference in the PV of males and females. This dimorphism difference in the PV is obvious from the beginning of juvenile age. The difference between males and females remains throughout every age; however, in old age the difference narrows.

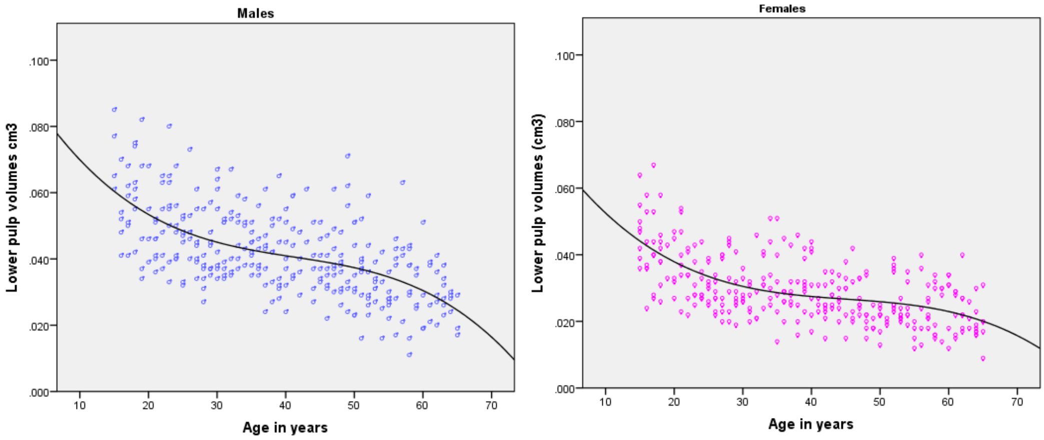


Figure 3.17 Scatter graph showing sigmoid S-shaped non-linear relationship between Model 4 and age.

The equation from the regression analysis derived for age estimation was as follows:

Estimated age = 60.370 + lower pulp volume × -715.260 + sex × 8.791.

Thus, the equation adds 8.791 to a person's estimated age when they are male and for sex, 0 for females, and 1 for males.

The "oddity" in the distribution of the scores is only visible because of the large sample size. When experiments were conducted repeating this process using a subset of our sample, the problems with non-linearity were obscured. This findings offers some explanation why researchers with smaller sizes may not have found a similar result. Given that applying a linear regression function to this clearly non-linear relationship is probably unwise, the descriptive statistics for mandibular volume in each group are reported here (Figure 3.17). Using these values, a basic calculator was created in Excel to assess whether a given score is consistent with membership of a given age group and sex. This calculator is included in the (Figure 3.18). The calculator includes the polynomial linear model but also includes the means and Standard deviation. By putting the calculated PV simply it calculates the region where estimated age falls in the expected 95 % of scores to fall (2 standard deviations on either side of the mean) for each age group and it checks whether a particular target score falls within that region.

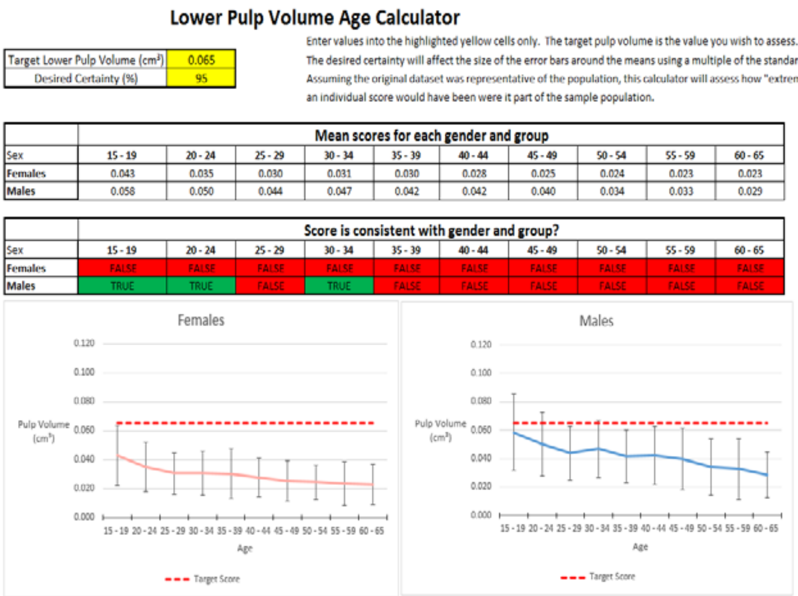


Figure 3.18 Age estimation calculator based on mandibular pulp volume.

3.6 Discussion

Despite having different contents and arrangements, pulp and dentine have a common embryonic origin. These two tissues share a close relationship in terms of physiologic and pathologic reactions. Anything that disturbs the dentine will affect the pulp, and vice versa (Mjor et al., 2001). Odontoblasts are the most prominent cells of the dental pulp derived from the dental papilla and are responsible for the formation of different types of dentine. Dentine is a mineralised tissue that surrounds non-mineralised dental pulp, and provides the long-living cells for dentine formation (Goldberg et al., 2011, Karjalainen, 1984, Sloan et al., 2015).

There are three types of dentine in a human tooth: primary, secondary, and tertiary (Goldberg et al., 2011). Primary dentine commences from odontogenesis until the tooth becomes functional and the formation of secondary dentine is produced immediately after primary dentine and continues throughout life (Sloan et al., 2015). Tertiary dentine is reactionary dentine which is laid down in response to an injury (Venkatesh et al., 2014). Secondary dentine formation increases with age thus, the volume of the pulp cavity shrinks. Therefore, researchers have calculated PV and utilised this as a predictor for estimating age (Ge et al., 2015, Ge et al., 2016, Andrade et al., 2019).

Very little was found in the literature on the question of secondary dentine formation. The relationship between secondary dentine formation and specific regions has seen conflicting interpretations in studies. Philippas reported that the site of secondary dentine formation is more in the floor of the pulp chamber than in the roof with age; whereas, Green reported that the site of secondary dentine deposition is larger in the roof of the pulp chamber than in the floor (Philippas and Applebaum, 1966, Green, 1955). Similarly, Murray et al. reported that an asymmetrical amount of secondary dentine was observed in the crown and root, with more secondary dentine formation noted in the root (Murray et al., 2002). These findings are contrary to those of Oi T et al., who used µ CT scans of extracted teeth and reported that no difference was found in the amount of secondary dentine formation between the floor and the roof of the pulp chamber with age (Oi et al., 2004). The cause of these different results could be the 2–D and 3–D studies.

Secondary dentine forms at a rate per day is 10 micrometres by odontoblasts, which then reduces to 4 µm/day when a tooth begins functioning (Goldberg et al., 2011). Bleicher. found that primary dentine forms at 4-8 µm/day, while secondary dentine forms at 0.5 µm/day (Bleicher, 2014). Age-related secondary dentine formation differences were observed between different teeth: 3.4% of dentine thickness was observed in the canine's crown and 15.5% and 34.1% in the crowns of incisors and premolars, respectively in old age (Murray et al., 2002). This rate of deposition of secondary dentine could be another reason for the discrepancy in the PV studies results.

A non-linear relationship between PV and age was found in this study. This result is in line with other studies results (Ge et al., 2015, Ge et al., 2016). A rapid formation of secondary dentine forms in early young age, then formation becomes slow and then become more rapid again in old age. This rapid decrease in PV in early young age is very much consistent with other 3–D studies (Ge et al., 2015, Ge et al., 2016, Oi et al., 2004). Regarding the relationship between PV and age, a sigmoid S-shaped linear relationship was found, which is different from other studies in shape but similar in terms of non-linearity (Ge et al., 2015, Ge et al., 2016). There is a strong possibility of non-linearity associated with a large sample size; however, the difference in the shape of function may be reflect a uniform distribution of the sample size, as other studies include only a small number of teeth from old age.

The present study's results reveal a significant difference in the PV of males and females. These results are similar to those reported by Zhi-pu et al. in 13 types of teeth, except for mandibular first molars (Ge et al., 2016). Similarly, other studies have also reported a difference in PV between males and females (Ge et al., 2015, Andrade et al., 2019). The results of this present study indicate a higher R^2 value than measured in single-root teeth. However, Zhi-pu et al. reported an R^2 value of 0.498 for maxillary second molars which is higher than in this present study (Ge et al., 2016). Furthermore, another study by Zhi-pu et al. reported a higher R^2 value for maxillary first molars, which is again higher than in this present study (Ge et al., 2015). This inconsistency in results may be caused by different methodologies and the teeth selected for PV measurements. Interestingly, this present study suggests that including sex as a predictor improves the predictive power of Model 4. It is apparent from (Figure 3.17) that sex-related differences were observed in the PV from the start of adolescence. Indeed, it appears it would be rather easier to estimate sex using PV than it is to estimate age. One possible explanation for this increased explanatory power might be the differences that exist between males and females in primary dentine formation. The evidence indicates that the Y chromosome controls the thickness of dentine; whereas, the X chromosome only controls the thickness of the enamel (Alvesalo, 1997). In addition, Zilberman et al. studied the sex and age-related differences in primary and secondary dentine formation in children. Their results indicate that the dimorphism difference found in the dentine thickness was even present in the initial stages of tooth formation and increased with puberty (Zilberman and Smith, 2001).

In this study, rapid formation of secondary dentine was observed in the mandibular canine until the 25-30 years old age group. In middle age, secondary dentine deposition slows and is consistent. In the 6th decade of age, rapid formation of dentine was once again observed. There is a significant difference between the PV of males and females and this difference remains throughout life. After 55 years of age, the difference narrows. It might be assumed, from this result, that in old age, the role of sex as a predictor becomes less informative.

One of the most notable differences observed in PV occurred in those over 55 years of age, this part of sample demonstrated a rapid reduction in PV in this study, which is contrary to other studies (Ge et al., 2015, Ge et al., 2016). This difference might be attributed to the homogenous age distribution with a large sample size. Again, discrepancies in the results of this study are most likely a product of the large sample size increasing the strength of this study to detect small and subtle effects.

There was very high variability in PV within each age group in this present study. These findings were observed in other studies also. The heterogeneity of PV was observed in every age group in this study. One possible explanation for the differences in the PV within a single age group in adolescence is that it may be at least partially attributable to yet unanswered factors that affect primary dentine formation.

It may be impossible to discriminate primary dentine from secondary dentine on CBCT images. Histologically, some researchers claim that the demarcation line between primary and secondary dentine makes them easy to identify (Karjalainen, 1984). Although there is not much difference between primary and secondary dentine, the significant difference is associated in the direction of S- curve shaped tubules (Karjalainen, 1984, Goldberg et al., 2011).

A study with a small sample evaluated the impact of periodontitis on PV. A comparison between healthy teeth and periodontitis without bone loss in the opposite tooth was made and the PV were evaluated. The results indicate that the mean volumetric difference was 19.1% between two groups of teeth, which is statistically significant ($p<0.05$). The study indicates that periodontitis causes a reduction in PV (Terlemez et al., 2018). On the other hand, the expression of oestrogen receptor (ER)-α in human pulps could be the reason for the difference in PV. The expression of (ER)-α is found more in the PV of females. This difference was more in the second phase of the menstrual cycle than other phases and menopause (Hietala et al., 1998, Jukic et al., 2003). Similarly, in the case of ovariectomy estrogen receptor (ER)-α enhance the secondary dentine formation in females (Yokose et al., 1998).

The changes in the homeostatic mechanism of pulp with age may be another factor for the variations in the PV. This mechanism means that PV decreases with age (Gomez and Cabrini, 2004). Many genes and proteins regulate the homeostatic mechanism, mineralisation, and vascularisation of dental pulp. Core-binding factor alpha -1 (Cbfa-1) is expressed in odontoblasts in the differentiation phase, from pre-odontoblasts to odontoblasts. Furthermore, dentine phosphoprotein and dentine sialoprotein (DSP) are also indicators for the differentiation of dental pulp cells. A significant difference was noticed in the expression of Cbfa-1, DSP and mRNAs between young and adult pulp. Young dental pulp cells expressed high levels of Cbfa-1, DSP and mRNAs compared with old pulps cells. This phenomenon means that large amounts of dentine formation are evident in young pulp compared to old pulp (Matsuzaka et al., 2008). It is possible that these factors could be the reason for the rapid formation of secondary dentine in young age that was observed in some studies (Ge et al., 2015, Ge et al., 2016, Kazmi et al., 2019).

Homeostatic mechanisms also reduce PV with age. This mechanism is maintained by angiogenesis. Some reports suggest that vascular Endothelial Growth Factor (VEGF) has a relationship with pulp and is an important regulator for vasculogenesis during embryogenesis and in the angiogenesis of adult tissues. Furthermore, VEGF is sensitive to stress, which stimulates its production; thus, it may affect the homeostatic mechanism. On the other hand, VEGF is expressed more in adult pulp than young pulp which might cause dental pulp stenosis with age (Matsuzaka et al., 2008, Yoshino et al., 2003).

There are other possible explanations for the PV inconsistencies. Venkatesh et al. suggest that PV are sensitive to orthodontic treatments (Venkatesh et al., 2014). In this present study, scans of unknown history were utilised for estimating age. Variations in the PV might be due to orthodontic treatment, especially in the early teenage years. Javed et al. evaluated the influence of orthodontics treatment on human dental pulp and suggest that the link between orthodontic forces and dental pulp tissues has been insufficiently validated. However, the study did not focus on pulp volumes as their primary outcome (Javed et al., 2015). Future research could further clarify the effect of variable magnitude and duration orthodontic forces on the volume of dental pulp tissue.

3.7 Summary of the chapter

- A strong relationship was found between maxillary and mandibular PV with age. However mandibular PV displayed a stronger relationship with age than maxillary PV.
- A non-linear negative sigmoid relationship was found between mandibular PV and age which is different from other PV studies. This could be due to large sample size and an even distribution of males and females.
- The nature of the distribution of PV suggests that this approach is most useful for age estimation with high or very low PV scores.
- Inclusion of sex as a predictor produced marginally better correlation with age.
- A statistically significant difference was found (p=0.000) between PV of males and females. Males are more strongly correlated with age than to females.
- A dimorphism difference exists from the juvenile age however in old age the difference narrow.
- An outcome of this study was described the secondary dentine formation with age. A rapid formation was observed until 25-30 years old, then a slowdown and consistency in middle age, followed by rapid formation after 55 years of age.
- A large sample with homogenous sample (even distribution across age groups) comprehensively explains the understanding of the PV with age.
- Based on the study findings, PV can be useful in young and old ages for age estimation.

3.8 Future work

- Inclusion of systemic history of the participants would help to determine the possible factors which influence the PV.
- Different methodologies using various teeth may improve age estimation.
- Magnetic resonance imaging techniques and others for PV might also bring the improvement in age estimation.
- Combination of different teeth and methods can be useful for estimating age.

Chapter 4. Age estimation using three–dimensional pulp tooth volume ratio

The introduction of 3–D techniques in dentistry, offered the possibility to evaluate tooth structure in three anatomical dimensions. These images not only overcome the limitations associated with 2–D images, but also provide excellent image quality. However, these images are quite costly and require special training (Shah et al., 2014, Patel, 2009).

Various 3–D techniques have been employed in dentistry for the diagnosis of dental diseases. These 3–D radiology techniques provide comprehensive knowledge of the internal structure of the tooth, which is important for successful dental treatment. Furthermore, these images provide opportunities for researchers to use them in their studies.

In dental research, a considerable range of techniques is available to visualise the tooth. Magnetic resonance imaging, computed tomography, spiral computed tomography, CBCT, µ-CT, synchrotron-radiation micro-computed tomography are used in dentistry for various research purposes (Kato et al., 2016). However, in forensic dentistry CBCT and µ-CT are commonly applied for facial reconstruction, sex, and age estimation.

In 2004, Saka et al. studied volume changes in pulp with age. The outcome of this research suggest that there is a volume reduction with age, which is more remarkable in young age (Oi et al., 2004). Similarly, in the same year, Vandevoort et al. calculated the PTVR of single rooted teeth with age using µ-CT and correlated it with age (Vandevoort et al., 2004). The results suggest a weak correlation with a linear relationship between them. On the other hand, Yang et al. used CBCT images and calculated the PTVR using single rooted teeth (Yang et al., 2006). Their results found a coefficient of determination of 0.29, with a linear relationship and 8.3 years of difference between estimated and chronological age. Furthermore, Tardivo et al. used CT scans of canines, and the outcome of the study suggests a non-linear relationship between PTVR and age (Tardivo et al., 2014). This discrepancy in results could be attributed to small sample size, as studies with small samples have reported a linear relationship between PTVR and age; whereas, medium to large sample sized studies suggest non-linear relationship between them. Additionally, most of the studies reported that there is no difference between PTVR of sexes with age. However, some studies have reported that a difference existed between their PTVR.

Therefore, to verify the usefulness of PTVR with age, a data set with a large sample size with a homogeneous age distribution is needed to explore the PTVR relationship with age and the accuracy of age estimation. In addition, the role of sex must also be evaluated as a predictor in age estimation.

4.1 Aim

To investigate the relationship between human canines, sex and chronological age.

4.2 Objective

To assess the relationship between the PTVR of the left maxillary and mandibular canines singly and collectively with and without sex as a predictor against chronological age using CBCT images of Pakistani subjects aged 15-65 years old.

4.3 Research questions

4.3.1 Question 1

Is canine PTVR, reliable predictor for age estimation?

4.3.2 Question 2

Are there any differences between the PTVR of males and females for estimating age?

4.4 Methodology

Ethical approval, sample size collection, and the calibration of the volume details provided in the chapter 3. The method for measuring PV was in chapter 3 therefore in this chapter the volumes of the tooth measurements are provided.

4.5 Slice thickness and slice interval

Slice thickness values are usually established by the investigator in accordance with the diagnostic task. It is recommended that, for minor and minute visualisation the slice thickness should not be increased. The image quality is impacted by the image noise which is related to the slice thickness. It is important to keep a balance between slice thickness, image noise and diagnostics task. Thinner slices increase the image noise, but the diagnostic information about the small lesion improves (Alshipli and Kabir, 2017). The results of the volume measurements showed that slice thickness up to 1mm can be used for the measurements (Chadwick and Lam, 2010). Similarly, the by increasing the slice interval up to 1mm can be chosen for the volumetric measurements (Sezgin et al., 2013, Kayipmaz et al., 2011).

To select the suitable slice interval, a small study was carried out with between 0.3mm and 0.5mm slice intervals. For this purpose, 99 teeth from 30 CBCT images were selected for the slice interval analysis.

4.6 Number of sections

The tooth was divided into 18 images so that all parts of the tooth are covered however in very few cases tooth was divided into 24 slices because of large size of tooth. The tooth appears in images 3 to 16. The initial and last images were kept empty, so that tooth is completely covered in the sections.

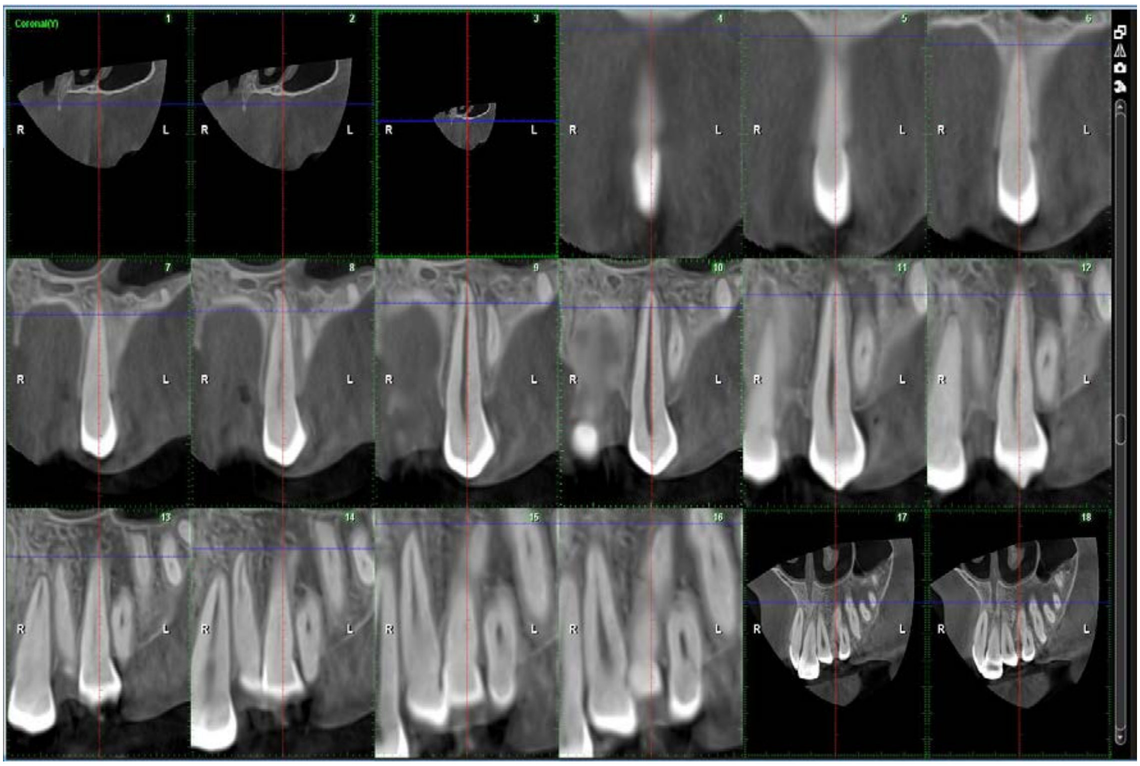
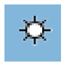
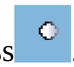


Figure 4.1 Tooth sectioning in coronal view.

4.7 Contrast, Brightness, Sharpness and Toggle Zoom

The range of 850- 1030 was selected for contrast  , 1820-2110 for brightness  , and 3-10 for sharpness. Each image was maximised  for better viewing and measurements.

4.8 Tooth Tracing

The ‘Free Region Grow’ icon  was selected from the annotation tools to trace the tooth outline. The outline of the tooth was drawn manually. A minimum of 20 points was used for the tooth outline. The dot placement begun from the mesial end of the root, followed the outline, and ended at the distal end of the root.

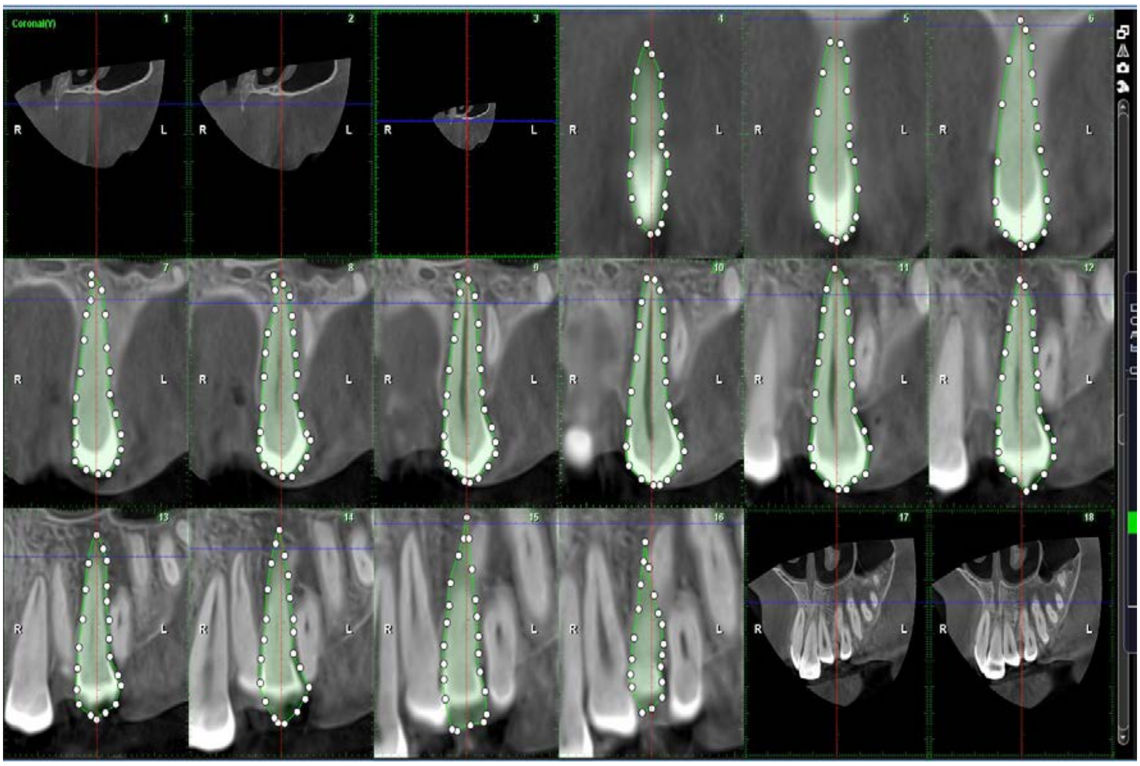


Figure 4.2 Tracing of the left maxillary canine outline in coronal view.

4.9Three–dimensional volumes of the tooth

After placing all the dots on the outline of the tooth, the ‘Create Region’ icon 

was clicked to obtain the volumes. A small window opened with the calculated volume.

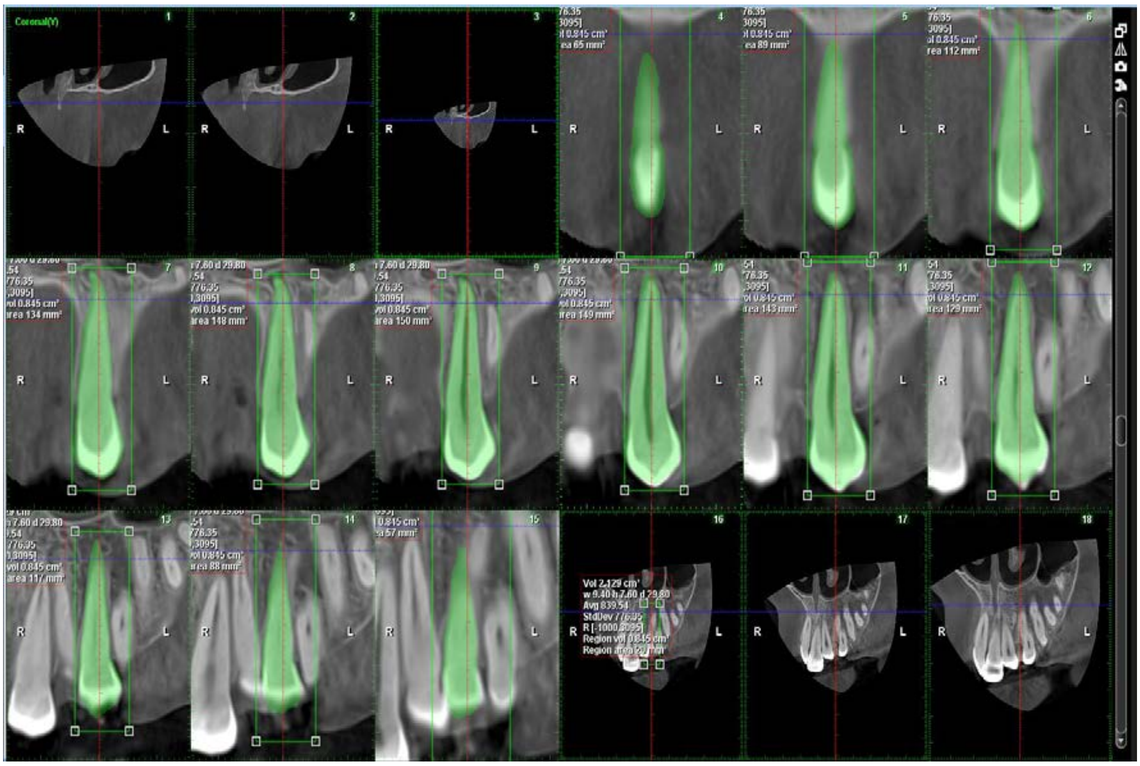


Figure 4.3 Obtained three–dimensional volume of the left maxillary canine.

4.10 Results

The correlation coefficient of slice thickness results ranged between 0.962 and 0.988 from 0.3 mm and 0.5 mm slice intervals (Appendix 7). These coefficient values are ≥ 0.9 which indicates a high correlation and strength between the values obtained from 0.3 mm and 0.5 mm slice intervals. Since values were high therefore, for ease, 0.5 mm slice intervals were selected for the tooth volume measurements.

Table 4.1 Correlation results between 0.3 mm and 0.5 mm slice thickness.

Teeth	N teeth ♂	Correlation Coefficient	N teeth ♀	Correlation Coefficient
Maxillary right canine	12	0.974	12	0.981
Maxillary left canine	14	0.962	11	0.988
Mandibular left canine	12	0.985	13	0.989
Mandibular right canine	12	0.985	13	0.986
Total	50		49	

4.10.1 Correlation between different tooth scores

A Pearson's correlation obtained values from left and right maxillary canine, left and right mandibular canine, left maxillary and mandibular canine and right maxillary and mandibular canine tooth volumes which yielded values between 0.871 and 0.966 (Appendix 8). These results indicate a close association between the teeth. Hence, the statistical analysis indicates that the comparisons between tooth volumes were not significant ($p>0.05$); thus, left maxillary and mandibular canine tooth volumes were selected for the volumetric measurements.

Table 4.2 r values by sex between left, right, upper, lower maxillary and mandibular canines.

Comparison between teeth volumes	N teeth ♂	r	N teeth ♀	r
Maxillary left and right	20	0.961	24	0.966
Mandibular left and right	33	0.947	26	0.959
Maxillary left and Mandibular left	22	0.931	21	0.907
Maxillary right and Mandibular right	21	0.959	25	0.871
Total	96		96	

4.10.2 Intra class correlation Results

Intra class correlation coefficient (ICC 2, 1- consistency) was performed to assess the test-retest and inter rater reliability.

4.10.3 Test–Retest reliability

The consistency of the measurements and the reliability of the measurements over time were assessed using test–retest reliability. All the values are above 0.9, which suggest high levels of repeatability and reproducibility (Appendix 9). Correlation Coefficient values by sex undertaken by the first observer

Table 4.3 Correlation Coefficient values by sex undertaken by the first observer.

Comparison between teeth volumes	**N teeth ♂**	**Correlation Coefficient teeth**	**N teeth ♀**	**Correlation Coefficient teth**
Maxillary left	25	0.979	27	0.978
Maxillary right	23	0.983	30	0.983
Mandibular left	35	0.981	30	0.980
Mandibular right	34	0.984	31	0.986
Total	117		118	

4.10.4 Inter–rater reliability

The level of agreement between the examiners was assessed using inter-rater reliability. High correlation coefficient values were obtained from inter-rater reliability which indicates a high degree of agreement between two examiners (Appendix 10).

Table 4.4 r values by sex between left, right, upper, lower maxillary and mandibular canines undertaken by the first and second observers.

Comparison between teeth volumes	**N teeth ♂ ♀**	**Correlation Coefficient teeth**
Maxillary left	23	0.985
Maxillary right	25	0.880
Mandibular left	31	0.931
Mandibular right	29	0.956
Total	108	

4.10.5 Inspection of normality

Prior to running any test, the data set was inspected for normality. A histogram was constructed to assess the shape and spread of the data. The results reveal that the data looked like bell-shaped displaying symmetry; therefore, the assumptions of normality were met.

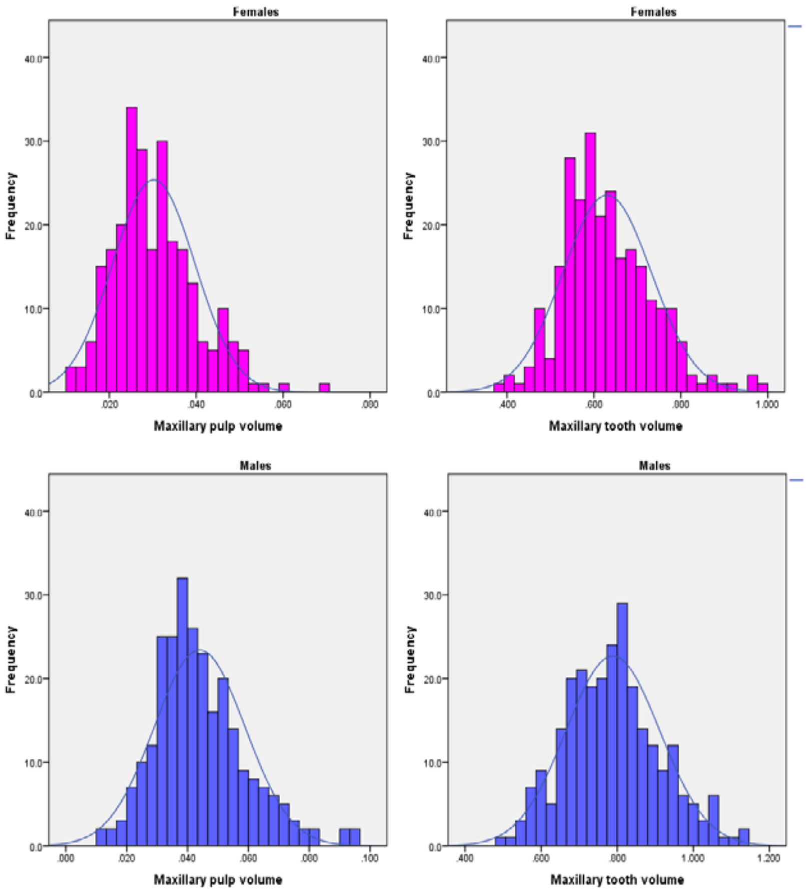


Figure 4.4 Histograms showing uniform pattern of maxillary tooth volumes.

4.10.6 Descriptive statistics results

A summary and description of the sample and its measurements are shown in the (Table 4.5).

Table 4.5 Descriptive statistics of left maxillary and mandibular canine by sex. TV= teeth volume. CI= confidence interval. SD=standard deviation. IQR= Interquartile range.

Gender	Scans	TV	N	Mean	95 % CI for mean		S. D	S. D		Range	IQR
					lower	upper		min	max		
Females	368	UTV	258	0.048	0.046	0.050	0.012	0.019	0.087	0.068	0.016
		LTV	313	0.054	0.052	0.056	0.013	0.018	0.098	0.079	0.018
Males	349	UTV	263	0.055	0.053	0.057	0.014	0.015	0.097	0.081	0.020
		LTV	307	0.060	0.058	0.062	0.013	0.025	0.096	0.071	0.019
Total	717		1141								

4.10.7 Formation of the regression models

All the calculated pulp volumes and tooth volumes were tabulated into the excel sheet against age and file numbers. An excel calculator was used to obtain the PTVR from pulp and tooth volumes. R statistical program and SPSS used for the further investigation. Six regression models with PTVR with and without sex were formed. The details of the models are provided into (Table 4.6).

Table 4.6 Six Models and predictors.

Models	Predictors
Model 1	Left maxillary PTVR
Model 2	Left mandibular PTVR
Model 3	Left maxillary PTVR and sex
Model 4	Left mandibular PTVR and sex
Model 5	Left maxillary and mandibular PTVR
Model 6	Left maxillary and mandibular PTVR and sex

4.10.8 Linear regression analysis

A step by step linear regression carried out between models 1–6 and age to assess the strength and association. The obtained values of the coefficient of determinations shown in (Table 4.7). The results indicated that all Models significantly correlated with age, but Model 3 showed that highest predictive power therefore Model 3 selected for further analysis.

Table 4.7 Regression values of the six Models.

Models	Predictors	R^2
Model 1	Left maxillary PTVR	0.44
Model 2	Left mandibular PTVR	0.42
Model 3	Left maxillary PTVR and sex	0.46
Model 4	Left mandibular PTVR and sex	0.44
Model 5	Left maxillary and mandibular PTVR	0.41
Model 6	Left maxillary and mandibular PTVR and sex	0.42

4.10.9 Diagnostic test and checking assumptions for Model 3

Diagnostic test and assumptions carried out for Model 3. Firstly, the studentized residuals calculated between expected and observed values. A graph plotted between residuals and fitted values and graph showed the evidence of non-linearity. Several transformations like square root, log, and reciprocal attempted to correct the non-linearity but none were found to improve it.

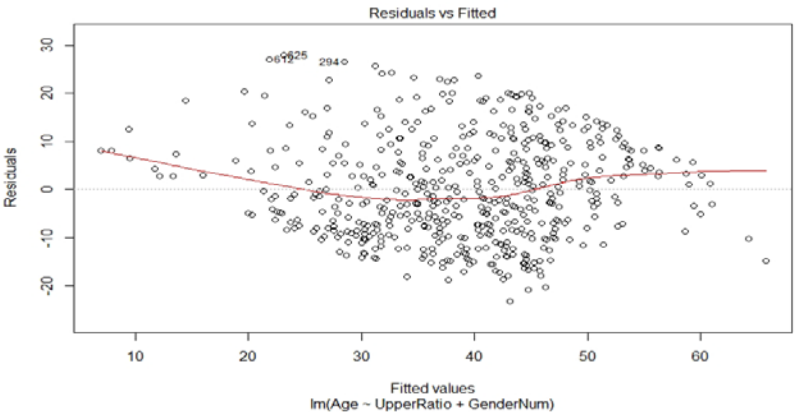


Figure 4.5 Plots of residuals against fitted values.

Model 3 was checked for the extreme studentized residuals scores. Hence values were +2.73 to -2.25 not exceeding ± 3 therefore it means that there were not any extreme scores.

Moreover, cooks distance was used to find the influential outliers in the predictors. No value ≥ 0.0223 were found, thus indicating that no individual score negativity affected the model causing bias to the results.

Similarly, a Durbin Watson test conducted to measure the autocorrelation in the residuals. Results showed that the p value was significant which indicated a serious problem with non-independence. The errors of Model 3 are not random: the predicted results estimated age young, which was actually older and vice versa.

In the end, multicollinearity was tested for highly correlated variables but there was not a problem with multicollinearity.

4.10.10 Poly nominal regression analysis of Model 3

The data has a slight nonlinear variation in the start and in the end. Since the straight line in the linear regression unable to capture the pattern of the data therefore to overcome the under and over fitting in the data poly nominal regression was carried out for Model 3. In order to generate Poly nominal regression equation, powers such as PTVR^2, PVTR^3 and PTVR^4 were added to the linear regression equation.

A poly nominal regressions (PTVR^2, PTVR^3 and PTVR^4) along with quadratic and cubic were tried. The quadratic and cubic models showed statistically significant improvement over the standard linear model. However it did still suffer from autocorrelation/non-independence, just like linear Model 3.

A cubic polynomial with the curve provided the better fit than the linear line. The cubic line looked best when fitted to the graphs, however when tried adding a cubic polynomial, it was not a statistically significant improvement over the quadratic polynomial. Additionally, the R^2 of linear model of Model 3 was 0.46 which also slightly increased to 0.48 in poly nominal regression.

4.10.11 Descriptive Statistics

The summary of the Model 3 provided into (Table 4.8).

Table 4.8 Descriptive statistics of Model 3 with age and sex.

	15-19 group	20-24 group	25-29 group	30-34 group	35-39 group	40-44 group	45-49 group	50-54 group	55-59 group	60-65 group
Counts										
Females	34	38	39	37	37	34	34	36	32	47
Male	31	37	39	35	40	29	37	34	33	32
Standard Deviation										
Females	0.007	0.008	0.008	0.009	0.008	0.007	0.007	0.009	0.010	0.008
Male	0.012	0.010	0.009	0.010	0.009	0.013	0.010	0.013	0.013	0.008
Means										
Females	0.067	0.061	0.050	0.051	0.048	0.044	0.044	0.040	0.041	0.034
Male	0.078	0.065	0.059	0.058	0.055	0.056	0.049	0.045	0.042	0.038

A scatter plot was used to express the relationship between PTVR and age. Results showed a clear non-linear relationship thus highlighting the problems related with trying to fit a linear line to this outcome. A non-linear relationship between PTVR existed. This non-linear relationship was found to be a non-consistent throughout the life. A rapid reduction was noticed in young age which becomes consistent in middle age and later becomes rapid again in old age. The innovative finding of this study is an S-shape non-linear relationship, contrary to the linear relationship reported by other studies. This S-shaped could be attributed to a large sample size used in this study.

A significant difference (p=0.000) was found in the PTVR of males and females. This difference existed from the start of the juvenile age and remained throughout the middle age but become narrow in the old age.

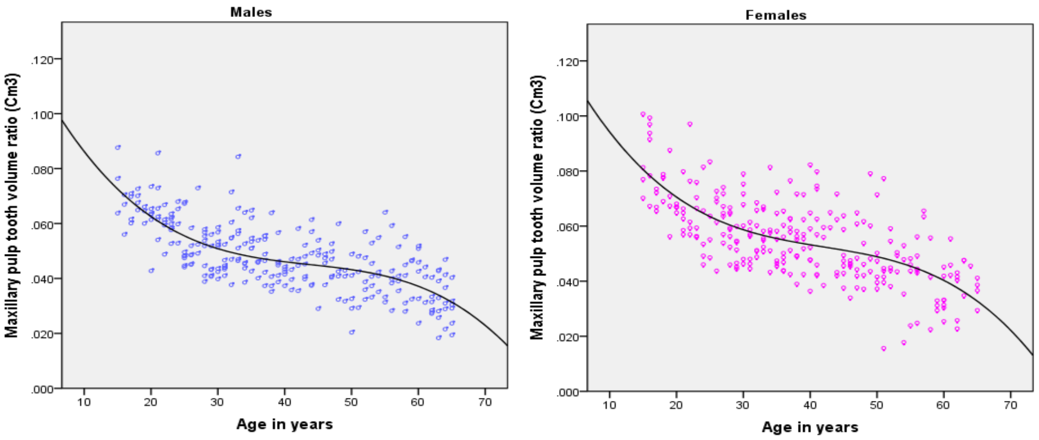


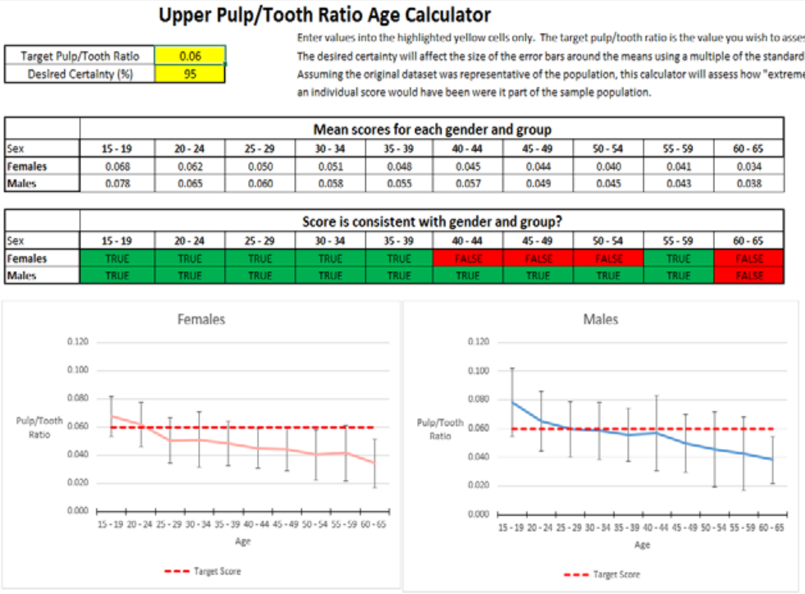
Figure 4.6 Scatter graph showing sigmoid S-shaped non-linear relationship between Model 3 and age

The equation from the regression analysis derived for age estimation was

Estimated age = 72.711 + upper PTVR × -691.426 + Sex × 3.8773.

The equation adds 3.8773 to a person's estimated age when they are males and for sex, 0 for females, and 1 for males.

The appearance of peculiarity in the distribution of results is only visible due to the large balanced sample size. Studies conducted with small sample size and uneven distributed the problems of the non-linearity are obscured. To somehow this explains the reason why researchers with small sample size could not identify this problem. The descriptive analysis of Model 3 provided into the (Table 4.8). Keeping in mind these values, a calculator has been developed in Microsoft Excel, which assesses whether a given score is consistent with the given group and sex. The details of the calculator provided into (Figure 4.7).



Upper Pulp/Tooth Ratio Age Calculator

Target Pulp/Tooth Ratio	0.06
Desired Certainty (%)	95

Enter values into the highlighted yellow cells only. The target pulp/tooth ratio is the value you wish to assess.
The desired certainty will affect the size of the error bars around the means using a multiple of the standard deviation.
Assuming the original dataset was representative of the population, this calculator will assess how "extreme"
an individual score would have been were it part of the sample population.

	Mean scores for each gender and group									
Sex	15 - 19	20 - 24	25 - 29	30 - 34	35 - 39	40 - 44	45 - 49	50 - 54	55 - 59	60 - 65
Females	0.068	0.062	0.050	0.051	0.048	0.045	0.044	0.040	0.041	0.034
Males	0.078	0.065	0.060	0.058	0.055	0.057	0.049	0.045	0.043	0.038

	Score is consistent with gender and group?									
Sex	15 - 19	20 - 24	25 - 29	30 - 34	35 - 39	40 - 44	45 - 49	50 - 54	55 - 59	60 - 65
Females	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE
Males	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE

Figure 4.7 Age estimation calculator based on maxillary pulp tooth volume ratio.

4.11 Discussion

A non-linear relationship was found between PTVR and age in this study, which is very similar to other 3–D studies (Sasaki and Kondo, 2014, Tardivo et al., 2014). PTVR decreases in young age, then becomes stable, and again rapidly decreases again in old age. Regarding non-linearity, this relationship is again very similar to other 3–D studies but different in shape, as this study found a sigmoid S-shaped relationship between PTVR and age (Sasaki and Kondo, 2014, Tardivo et al., 2014). There is a high probability that this non-linearity relationship is due to the large sample size, as this study and other 3–D studies have all used large sample size. However, the difference in shape could be due to the balance in the number of individuals in each group and age range of this study, as other 3–D studies lacked a uniform distribution of the sample size, especially in old age.

A significant difference was found between the PTVR of males and females which is very similar to other studies but different in terms of sex. This study found that males are highly correlated with age, but mostly other studies reported that females are closely correlated with age. As is evident from the (Figure 4.6), the difference between the sexes was apparent from a young age. The significant difference between the PTVR of males and females remains consistent throughout life. The difference narrows after the age of 55; therefore, it can be supposed that the role of sex as a predictor is less helpful in old age.

Interestingly, this present study suggests that including sex as a predictor improves the predictive power of Model 3. Significant differences between real and estimated age, with various ranges of coefficients of determination of age were found by researchers. Regarding coefficient of determination, this present study found a higher R^2 value than other CBCT studies, but lower than in µCT studies. These result can be attributed not only to a large and uniform distribution sample (even distribution of age), but also to other factors. For coefficients of determination, the obtained results from µCT are superior to CBCT. µCT provides images with a higher resolution than CBCT. This result enables us to measure pulp tooth volumes more accurately, especially the border edge between the pulp and dentine. However, due to the high radiation dose, longer scan time, and the need for an extracted tooth, these factors make the process unusable in living individuals.

There was a diverse variety of PTVR within each age group in this present study, which was also observed in other studies. The patterns of tooth size and dental dimensions vary between different ethnic groups. Regarding variability and patterning, significant differences in mesial–distal crown dimensions and patterns of crown sizes were observed. In relation to mesio-distal and buccolingual crown dimensions, the largest teeth were from Australians. Regarding tooth size, western Europeans have the smallest teeth (Hanihara and Ishida, 2005). Similarly, differentiation within and diversity among the population can be due to environmental causes. Tooth wear occurs with age and contributes to the differences in tooth dimensions. There are specific factors that can affect tooth wear, such as nature of the diet, masticator forces, non-chewing parafunctional activities, and usage of teeth as a tool. A potential contributor to tooth wear is a tough fibrous diet, which requires prolonged mastication, especially in old age. These factors reflect the contribution of genetic and environmental influences to tooth size within and between different populations (Brook et al., 2009).

Using various types of teeth could be another reason for the diversity in the statistical correlation results. Additionally, differences in tooth shape and size cannot be ignored. Mandibular central incisors were selected due to having the lowest morphological diversity in human permanent teeth (Fuller, 1984).

Using PTVR from multiple teeth or a single tooth as predictors is also a matter of concern. Star et al. used anterior teeth collectively and individually, and their results suggest that individual teeth provide a higher coefficient of determination (Star et al., 2011). However, Nima et al. results suggest that there is no difference in the predictive powers of using teeth collectively or individually for age estimation (Biuki et al., 2017). Regarding, the comparison between different teeth and age, maxillary central incisor was found the highest correlation with age (Gulsahi et al., 2018, Biuki et al., 2017, Haghanifar et al., 2019, Asif et al., 2019).

The pattern of secondary dentine is not laid down homogenously in the pulp cavity; it differs in relation to bucco-lingual and mesial-distal widths and in incisal apical direction. Someda et al. measured different regions of the tooth and found higher correlations between whole PTVR and age (Someda et al., 2009). In contrast, to these findings, Asif et al. found that pulp chamber volume and crown ratio provided a higher coefficient of determination value (Asif et al., 2018). Similarly, Aboshi et al. measured secondary dentine at different levels, and their results suggest that the coronal-one third of the root is highly correlated with age (Aboshi et al., 2010). Thus, measuring these specific sites can carry variations in the correlation results.

Statistical consideration is very important for data analysis. Statistical power analysis should be considered before the start of a study. It is the probability that the test will detect an effect that actually exits. It gives the estimated observations that researcher need to have a good chance of detecting the effect looking for. In power analysis, usually four parameters (sample size, effect size, significance level, and power) are involved.

Sample size is the number of observations in the sample. Effect size is the quantified magnitude of a result present in a population. It is the measure of the strength of the relationship between two variables and can be measured using Pearson's correlation coefficient as a specific statistical measure. The larger the effect size, the stronger will be the relationship between two variables. The effect size is the minimum deviation from the null hypothesis that researcher hope to detect. Significance level is nominated with alpha and often set at 5 % or 0.05. Alpha is the significance level of the test (the P value), the probability of rejecting the null hypothesis even though it is true (a false positive). Finally, statistical power or beta is the probability of detecting effects that actually exists. All four variables are related to each other. Statistical power analysis is a powerful tool for design, analysis, and is perhaps most commonly used to estimate the minimum sample size required for the experiment (Thomas, 1997).

Another interesting confusion is often between the power analysis and statistical significance. Statistical significance is the comparison between two groups to find any differences. If the found differences are large, then this is considered significant; if they are small, then they are not considered significant. Regarding results, statistical significance is superseded by statistical power. For example, a study with a confidence level of 2.0 mm has a statistically significant difference of 0.5mm between two groups, so this findings would not be much helpful, since 0.5 mm is less than the study's power of 2.0 mm (Thomas, 1997, Molen, 2010).

The obtained results are highly influenced by the age distributions of the sample. Bocquet-Appel and Masset were the first to point out that a uniform and wider distribution of age range is an important factor in paleo demographic studies (Bocquet-Appel and Masset, 1982). Similarly, Rolseth et al. suggest that bias such age mimicry can occur if studies do not consider the balance in the number of individuals in each group and the age range of the study. Additionally, this problem could not be overcome due to a low correlation between age estimation indicators and age (Rolseth et al., 2017).

Presently CBCT units produce state of the art images, with excellent resolution and low radiation, providing greater clarity of 3–D anatomy images of the mouth for treatment. However, there are inherent factors in CBCT units related to technical aspects that can affect the nature of the image acquisition and reconstruction process. These factors could be the reasons for the inaccuracies and variations in results; therefore, it is important to be aware of these factors. There are some qualities of the scanned image that are interrelated and that can affect each other. Additionally, these factors determine the displayed image quality.

One possible explanation concerns the exposure parameters used to scan the tooth. The exposure settings used during the image acquisition such as tube current, tube voltage, filtration, collimation and focal spot impact the image quality and, thus, affect the diagnostic accuracy.

Conflicting results are associated with the effect of exposure parameters on objects. Likubo et al. evaluated the effect of exposure parameters (tube current and tube voltage) and gutta-percha cone on fracture-like artefacts on CBCT images. To achieve the minimum number of artefacts, higher tube voltages and lower tube currents are recommended (Iikubo et al., 2019). Similarly, Jasa et al. suggest that unclear radiolucent structures require higher exposure parameters (tube current and tube voltage); whereas, low exposure parameters can be used for clear radiolucent structures (Jasa et al., 2017). Regarding the effect of exposure parameters on bone surface and volume, it was found that these are not affected by tube current if the radiation dose is kept constant (Pauwels et al., 2015b). Furthermore, Wang et al. suggest that gradually increasing the kilovoltage from 40 to 55 KV and the milliampere from 0.4 to 4 mAs improves the image quality. Above that this value of parameters, the image quality begins to deteriorate (Kei Ma et al., 2014).

Increasing the tube voltage and tube current reduces the image noise but increases the radiation dose to patients (loubele 5). It is generally agreed that ALARA (as low as reasonably achievable) should be maintained with the diagnostic task. These exposure parameters can be considered influencing factors and remain quite challenging to balance during a CBCT scan. It is the radiologists and operators’ task to determine the settings, depending upon the diagnostic task, as these factors are related to image quality (Katkar et al., 2016). These factors could be the some of the reasons for the variations of age estimation results, because the used scan in the age estimation studies are not scanned truly uniformly because of different diagnostic tasks. In addition, a wide range of CBCT machines with different operational settings is used for images; thus, the scanned images undergo different exposure parameters values.

There are pulsed and continuous exposures in the X-ray tubes of the CBCT. Pulsed exposure ensures that there is no exposure between projections. As a result, there is a large discrepancy between scan time and exposure time. Conversely, in a continuous exposure, scan and exposure time are the same. The advantage of the pulsed exposure is that it may exhibit improved spatial resolution. Other factors such as the rotation of the x-ray projections of CBCT, can affect the image quality also. Partial rotation of the rotation arc 180 is associated with reduced dose and higher noise than a 360 rotation (Pauwels et al., 2015a).

Dose is defined as the product of the tube current and exposure time. Dose varies depending upon the patient size and desired image quality. Dose has a relationship with imaging parameter. Increasing the dose, affects the imaging parameters, similarly, increase in FOV size, tube current, tube voltage and voxel size also associated with high dose (Jasa et al., 2017, Pauwels et al., 2015a).

A location where accelerated electrons collide is called a focal spot (White and Pharoah, 2014). Smaller and broader size focal sizes are usually available in CBCT, with smaller focal sizes providing a higher resolution which is one of the important determinations of image sharpness. Regarding the impact of focal size on radiologic image quality, Gorham et al. state that no significant differences were found between images recorded with focal sizes of 0.8 and 1.8 mm (Gorham and Brennan, 2010). Similarly, Wang et al. found that no significant differences was in image quality between small and large focal sizes recorded with different tube and current voltages (Kei Ma et al., 2014). Finally, Ruben et al. suggest that improvement in the image quality can be achieved with small focal spots, if all parameters of the imaging chain are optimised as well (Pauwels et al., 2015a).

Unsharpness is defined as boundaries between dark and light area and ill-defined area, resulting in a blurred edge and known as unsharpness. There are four types of unsharpness; -

- Geometric unsharpness
- Image receptor unsharpness
- Movement unsharpness
- Edge unsharpness

If the detector lies across the light and dark border, the voxel will have an average value of these two values; - thus, a blurred border is created. If a tapered edge object moves during the acquisition, the tapered edge side of the object will be blurred, creating unsharpness, and there will be a decrease in attenuation along the object. If not, all photons pass through the object, this will cause a penumbra. If the scanning object is placed close to the focal spot, this will increase the penumbra as a result of unsharpness. ‘Source to object’ and ‘object to detector’ are important factors for determining the sharpness of the images. These distances vary considerably between scanners. These geometric factors can cause unsharpness at the edges of the images, creating a penumbra. A smaller focal spot, larger SOD and smaller ODD are all responsible for the decrease in the penumbra, which ultimately increases the image sharpness (Pauwels et al., 2015a).

Magnification is related to the distances between the object, focal spot, and receptor. Distortion depends on the angle that the beam passes through an object; therefore, it can distort the shape of the object (Pauwels et al., 2015a).

Differences in the image quality between detectors could be another reason for the variations of the results. X- Ray detectors convert the incoming photons into an electrical signal. CBCT machines mainly have either a charge-couple device (CCD) or flat panel detectors (FPD). Charge-couple device detectors are larger, more sensitive, and susceptible to distortion from a magnetic field. Therefore, distortion is created when the image gird moves away from the centre. Additionally, phosphors in image intensifiers lose sensitivity over time, and need to be replaced to maintain image quality. The flat panel detector is thin, small, and less bulky in nature. The images acquired from these panels have minimal distortion; thus, they generate better image quality (Abramovitch and Rice, 2014).

Reconstruction algorithms are used to reconstruct an image from multiple projections. In general, these can be divided into three groups: filtered back projection (FBP), algebraic reconstruction techniques (ARTs) and statistical methods. Due to its simplicity and rapid reconstruction of images, the Feldkamp-Davis-Kress (FDK) algorithm in the FBP group is commonly used in almost all CBCT machines. The algorithm consists of ramp and smoothing filters, which are used in the final reconstruction of an image. The smoothing filter helps to reduce the high-frequency noise associated with ramp filter and can significantly affect the image quality by reducing this noise. These filters use cut-off frequencies, which are usually expressed as a fraction of the Nyquist frequency. If the cut-off frequency is high, then both the spatial resolution and noise will increase; - as a result, the reconstructed image will be sharper but nosier (Pauwels et al., 2015a, Scarfe and Farman, 2008).

Spatial resolution is defined as the minimum distance needed to discriminate between two objects. The two objects must be seen separately, and detectors must be able to identify the distance between them. It is possible that larger detectors cannot identify a gap between two objects, therefore, they are seen as one object. However, smaller detectors can easily identify gaps between two objects, so display them as separate. Additionally, detector size thickness matters for image quality. The wider the detector row, the lower the resolution and the better the image quality. In addition, focal size spot, smoothing filter and reconstructed voxel size are other factors that determine spatial resolution. Partial volume averaging, noise, and artefacts are factors that make it difficult to obtain a resolution equal to the voxel size (Katkar et al., 2016, Molen, 2010, Scarfe and Farman, 2008, Pauwels et al., 2015a).

Partial volume is an important factor influencing spatial resolution and is defined as a voxel size larger than the object or its densities. This occurs frequently along the boundary of an object or at the margins of two substances with different densities. Due to this phenomenon, the voxel displays an average of the densities. For example, if the voxel represents 75% pulp and 25% dentine, it will appear more lucent than opaque. This makes boundaries difficult to distinguish and leads to lower spatial resolution. The influence of partial volume can be reduced by decreasing the voxel size; however, this results in more radiation and is more prone to noise and poor spatial resolution. The results of the partial volume effect on the accuracy of tooth volumetric measurements using CBCT is controversial. Some researchers suggest that the process leads overestimation, while others think it causes the underestimation of the true tooth size (Molen, 2010, Spin-Neto et al., 2013, Ye et al., 2013, Peters and Maret, 2013).

Noise is the haphazard variation of voxel values in an image or can be defined as unintended photons hitting the detectors and resulting in a clouded image or random variation in the photon numbers forming the image called noise. There are different types of noise that arise from different sources in radiographic images, but mainly quantum, electronic and noise introduced by the reconstruction process that is back projection are common sources of noise.

This noise hinders the signal received from the tooth. The level of noise varies between the machines. Image noise is affected by the reconstruction software and machine settings. Higher noise in the image obscures the contrast between the objects (Scarfe and Farman, 2008, Pauwels et al., 2015a). Image noise can also affect both contrast and spatial resolution.

Scatter radiation is the main cause of noise in a scan. These levels are associated with the size of the field of view (FOV) because as the size increases, scatter levels also increase. These larger FOVs increase the amount of scatter radiation reaching the detector, eventually increasing noise and artefacts. In addition, larger FOVs are associated with higher radiation doses and worse spatial resolution. Therefore, a large FOV scan should be avoided when evaluating pulp tooth volumes (Molen, 2010). The quality of the image can be improved by reducing the noise, which can be achieved by increasing the number of photons detected or formed in image.

Image quality can be evaluated subjectively and objectively. Subjective evaluation of the image is related to the observer's opinion on the clarity of the visualization of an anatomical structure. Contrast-to noise ratio (CNR) is widely used to determine image quality measurements and as an objective measure of image quality. The CNR is based on image contrast rather than the raw signal. During the image acquisition, head position can affect the image quality. Tilting the head backwards especially results in a higher CNR value in the mandibular region, regardless of the FOV or CBCT machine used. Similarly, increasing the number of basis images and tube current significantly contributes to CNR increase (Pauwels et al., 2015a, Katkar et al., 2016, Molen, 2010, Lindfors et al., 2017).

There is always some background noise in CBCT. To obtain a good image, the level of signal from the imaged should be greater than the noise. More signal than noise means a higher signal-to-noise ratio SNR and a better and more useful image. A higher SNR means better and more useful image (more signal than the noise). The greater the voxel size, the greater will be the signal in the image and the better SNR. However, a greater voxel size in image results in lower resolution.

To improve the signal, scan the image several times. Number of projections is also associated with resolution as larger number of projections provides finer resolution.

Voxel size is the smallest 3–D element of the volume, usually represented by a tinny box or cube shape with height, width, and depth. Voxel resolution is the size of the voxel (Scarfe and Farman, 2008). Each voxel has grey scale value that depends on the attenuation of the material inside it. The image quality depends on resolution size. The smaller the voxel, the greater the voxel resolution. Voxel resolution is a possible factor influencing image quality. Isotropic voxel size reduces the partial volume effect and provides better multi-planer reformatting (Pauwels et al., 2015a).

Smaller the voxel size, the greater will be the noise but higher spatial resolution. The size of the voxel size varies according to the diagnostics task, but no protocols have been set for the particular diagnostics tasks in dentistry (Spin-Neto et al., 2013). Regarding the diagnostic ability of CBCT images and voxel size, the results suggest that diagnosis was easier with 0.2 and 0.3mm voxel size than with 0.4mm (Librizzi et al., 2011). Therefore, voxel size might not be constant for every patient, but depends on the diagnostic task. Regarding the accuracy of linear measurements, no difference was found between the 0.2 and 0.4mm sizes (Damstra et al., 2010). Regarding, tooth volume measurement, studies have found that measured tooth volumes with CBCT of 200mm and 300mm were underestimated when compared with CBCT 76mm voxel size and µ-CT 41 voxel size (Maret et al., 2012). However, these results are contradicted by Ye N et al., who found tooth volume measurements tended to be overestimated (Ye et al., 2013).

Voxel resolution can be an important factor affecting volumetric measurements. Different voxel sizes were used for pulp tooth volume measurements because CBCT scans with different voxel sizes were used according to the diagnostic needs. Asif et al suggest using 0.3mm voxel size to nullify the partial volume averaging effect of voxels on volumetric measurements (Asif et al., 2018). Maret et al. findings suggested that measurements made using of voxel sizes ≥ 0.3mm are significantly underestimated (Maret et al., 2012). Waltrick et al. suggest that a 0.3mm voxel size is perfect and good compromise for radiation dose and image quality (Waltrick et al., 2013). Similarly, Damstra et al. state that no improvement was evident in the accuracy of measurements by increasing the voxel size from 0.4mm to 0.25mm (Damstra et al., 2010). Adisen et al. results also suggest that no statistically significant difference was found in the measurements of 0.2 and 0.4mm voxel sizes; thus, 0.4 mm can be used for age estimation (Adisen et al., 2018). Similarly, evaluating linear bone measurements, no differences were found between voxel sizes of 0.2, 0.3 and 0.4.

The gray scale bit depth of the CBCT system is also related to the image quality. Bit depth is another property of the image detector, an exponential binary property expressing the total number of gray shades that a detector can discriminate. Each voxel size has a grey scale value assigned during the image reconstruction phase. Current CBCTs range from 12 bit to 16-bit gray scale. The human eye cannot distinguish beyond 10-bit gray scale, and available computer monitors are currently available in only 8 to 10-bit gray scale. It is recommended that when evaluating the small structures, the highest available gray scale should be used (Pauwels et al., 2015a, Molen, 2010).

Field of view (FOV) is the volume being imaged or the anatomical area that will be irradiated. It determines the area to be imaged. The FOV can be classified into small, medium and large according to the type of the detector used for the CBCT scan (Abramovitch and Rice, 2014). Regarding the influence of small, medium, and large fields of view on the accuracy of the linear measurements, the results suggest that smaller FOV's are associated with higher measurement accuracy; - however, both medium and large FOV's displayed a statistically significant difference (Elshenawy et al., 2019).

Slice thickness is the distance between the start plane and end plane of a single slice; whereas, interslice interval is the distance between the start planes of two consecutive slices, which can affect the image quality of the CBCT. Thinner slices provides better resolution hence increases the visibility of the small lesions to achieve more diagnostic information but are associated with worse noise (Alshipli and Kabir, 2017). Conversely, thicker slices decrease the image noise but increase the partial volume effects. Image smoothness, reduction in noise and sharpness can be achieved by increasing the slice thickness (Molen, 2010).

Regarding the bone height measurements, significant differences were found between bone heights and slice intervals when these were greater than 1mm (Chadwick and Lam, 2010). In terms of bone defect volume, slice thickness and an interval up to 1mm can be selected for the volume calculations on CBCT images (Sezgin et al., 2013). Similarly, the volume of the defects in the bone were measured up to a 0.4mm slice thickness and interslice interval; the, results did not find any difference up to 0.4mm (Kayipmaz et al., 2011). Pour et al. found no significant difference in the visibility of inferior alveolar canal up to 2mm slice thickness and interval (Pour et al., 2016).

The effect of window width and level can also affect image quality. The window /level are adjusted to obtain a suitable image contrast for the visualisation of the anatomical structures. Window width is the grey scale values available for display; the value below the lowest grey scale being displayed as black, and the highest as white. The window level determines the central grey scale value within the window width. A large width window covers the entire grey scale value of the image, meaning poor image is contrast achieved; whereas, a small width window leads to high contrast. However, a medium width window creates an overall good contrast (Pauwels et al., 2015a).

The monitor display requirements of the monitor have a limited and secondary role because of the use of the window/level and zooming tools. However, the size and resolution of the monitor should be considered while viewing digital images. The images should be displayed in their native resolution which is a 1:1 ratio between image and display pixel. An image with a voxel size of 0.2mm with a 50 ×50 mm CBCT image means every slice in this image will have 500 ×500 pixels. To visualise this image, a monitor with a minimum resolution of 1000 ×1000 pixels is required to display each slice with a 1:1 ratio. To avoid the bottleneck of the image sharpness, large, high resolution monitors are advised, as well as zooming tools and maximising the windows applicability (Pauwels et al., 2015a).

Comparison of the accuracy of the measurement tools of the different available software is an interesting and vital factor. Regarding vertical bone linear measurements from CBCT, no significant difference was found between the measurements from different software and the gold standard (Linear measurements of bone with a digital calliper). However, the smallest difference from the gold standard was found using On Demand3D and KDIS3D and the greatest difference was found using from XoranCAT.

Radiographic image contrast concerns distinguishing between two materials or tissues of different densities. Image contrast is the density difference between two materials or tissues of different densities on the image or can be. The mean difference in voxel value between two regions of the image (e.g. the mean difference between pulp and tooth). It also depends on factors such as exposure, the dynamic range of the detector and bit depth. Similarly, perceived image contrast depends on display settings, such as window/level. A high contract image possesses a larger difference between the displayed grey scale shades but has a smaller range of greys. Conversely, a low contract image possesses a small difference in the displayed grey scale shades and thus, it is more difficult to have different areas but has a larger range of greys.

Subject contrast concerns the differential attenuation by the tissues in the subject being imaged. A narrow and wide window can alter the image contrast on the monitor. A narrow window results in a larger difference in the grey value between the Hounsfield units; whereas, a larger window possesses a small difference in the grey value (Pauwels et al., 2015a).

4.12 Summary of the chapter

- The PTVR of maxillary and mandibular teeth displayed a strong relationship with age. However, maxillary PTVR displayed a stronger relationship with age than mandibular PTVR.
- Maxillary PTVR displayed a non-linear negative sigmoid relationship with age. This could be due to large sample size and an even distribution of males and females.
- The nature of the distribution of PTVR suggests that this approach is most useful for age estimation with high or very low PTVR scores.
- Inclusion of sex as a predictor produced a marginally better correlation with age.
- A statistically significant difference was found (p=0.000) between in PTVR of males and females. Males are more strongly correlated with age than females.
- A dimorphism difference exists from the juvenile age; however, in old age, the difference narrows.
- A rapid formation was observed until 25-30 years old, then a slowdown and consistency in middle age, followed by rapid formation after 55 years of age.
- A PTVR can be more suitable for age estimation for young and old ages.

4.13 Future Work

- Population specific formulas are recommended to estimate the age
- Different methodologies with various teeth are recommended to improve the age estimation.
- Using micro-CT technique and others for PTVR might also improve in age estimation.
- Due to the nature of secondary dentine deposition, it is more helpful in the early young age and old age, therefore, studies are recommended on this specific age groups.
- Inclusion of systemic history of the participants such as life style, toxic habits, diseases, and treatments will be helpful to find out the causes of variabilities in results.
- Combination of different teeth and methods can be more useful for estimating age.

Chapter 5. Conclusion

Both 2–D and 3–D images have been widely used in non-destructive methods measuring the size of pulp and tooth. Radiographic methodologies applied to 2–D and 3–D images are the same which are based on correlations between age and ratios of the width, height, various pulp tooth locations, and whole pulp tooth.

In the literature, dental age has been successfully estimated from 2–D images by using the ratios between pupal size and tooth size. However, these 2–D images are the accumulation of horizontal or parallel aspects of the tooth, therefore, the ratios calculated from measurements never represent the entire 3–D morphological changes in teeth.

The introduction of 3–D images in dentistry has provided detailed evaluation of teeth from a 3–D perspective, specifically the enamel, dentine and pulp cavity. Compared with CBCT, μ–CT provides better 3–D volume information. Regression analysis results from PTVR using μ–CT is likely to yield a better result than CBCT but requires extracted teeth and so is not a useable modality in a clinical dental setting.

Alternatively, 3–D CBCT images of living individuals can be acquired from dental archives. Thus, providing the opportunity for volumetric studies for dental age estimation. The present study investigated the relationship between PV, PVTR, and age among Pakistani adults. The results indicated that a strong relationship exists between mandibular canine PV, maxillary PTVR, and age. However, maxillary PTVR provided a higher strength of correlation values with age as compared to mandibular PV. An odd S-shaped non-linear relationship was found between PV, PTVR, and age but was only evident secondary to the large sample employed. Moreover, including sex as predictor improves the predicative power.

The conclusion is that using predictors such as mandibular PV and maxillary PTVR with sex produced the best estimates of age. CBCT volumetric measurements of various parts of tooth can be useful for dental age estimation in living subjects.

Appendix 1: Ethical approval to use the data for research

Advanced Digital Imaging

Dr. Ghazala Wali
MBBS, MCPS, FCPS
Radiology
Consultant Radiologist

Certification of Approval

Letter No 16062017/2

We Advanced Digital Imaging on the recommendations of Ethical Review Committee hereby approve sharing CBCT datasets for the following research.

Name of Project	Age estimation by tooth volumes using CBCT images
Head of project	Dr ShakeelKazmi PGR University of Dundee
Affiliation	Riphah university Pakistan
Approval includes	Project outline submitted 6th December 2016
Approved by	Dr.GhazalaWali
Date of approval	10th December 2016
Date of expiration	10th December 2018

The project have been reviewed and approved according to the Declaration of Helsinki by Ethical Review Committee.

The CBCT datasets for the above mentioned research will be anonymised and no personal information of Patient or prescribers will be provided except age, gender, date and time of acquisitions.

Ghazala
Dr.GhazalaWali
Chairman of the ethical Review committee
Advanced Digital Imaging
Dated;- 16-06-2017

Zulfiqar Ahmad Atiq
Managing Partner
Advanced Digital Imaging

16/6/17.

CT Scan | Open MRI | Digital X-Ray | 3D CBCT & OPG | Colour Doppler & Ultrasound | Laboratory

Appendix 2: Permission from NHS Tayside to use sample for research

Catrina Forde (Staff)
Tue 05/12/2017, 12:49
Shakeel Kazmi (PG Research); elivingstone@nhs.net

Reply all

Inbox

Hi Shakeel,

If you have received the data from India with appropriate ethical approval and in compliance with their Data Protection regulation, there is no issue with using it.

I assume you are not accessing/using NHS Tayside data in your project?

Regards,

Catrina

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Senior Clinical Research Governance Manager

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TASC
TAyside medical Science Centre

NHS Tayside

Appendix 3: Correlation scores of maxillary left and right, mandibular left and right, maxillary left and mandibular left and maxillary right and mandibular right canine pulp volumes in males and females

Males **Females**

Pearson Correlation between maxillary left and right pulp volumes in males and females

Correlations (Males)

		p1	p2
p1	Pearson Correlation	1	.943**
	Sig. (2-tailed)		.000
	N	25	20
p2	Pearson Correlation	.943**	1
	Sig. (2-tailed)	.000	
	N	20	23

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations (Females)

		p1	p2
p1	Pearson Correlation	1	.914**
	Sig. (2-tailed)		.000
	N	27	24
p2	Pearson Correlation	.914**	1
	Sig. (2-tailed)	.000	
	N	24	30

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between mandibular left and right pulp volumes in males and females

Correlations (Males)

		p3	p4
p3	Pearson Correlation	1	.957**
	Sig. (2-tailed)		.000
	N	35	33
p4	Pearson Correlation	.957**	1
	Sig. (2-tailed)	.000	
	N	33	34

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations (Females)

		p3	p4
p3	Pearson Correlation	1	.944**
	Sig. (2-tailed)		.000
	N	30	26
p4	Pearson Correlation	.944**	1
	Sig. (2-tailed)	.000	
	N	26	31

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between left maxillary and mandibular pulp volumes in males and females

Correlations (Males)

		p1	p4
p1	Pearson Correlation	1	.925**
	Sig. (2-tailed)		.000
	N	25	22
p4	Pearson Correlation	.925**	1
	Sig. (2-tailed)	.000	
	N	22	34

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations (Females)

		p1	p4
p1	Pearson Correlation	1	.844**
	Sig. (2-tailed)		.000
	N	27	21
p4	Pearson Correlation	.844**	1
	Sig. (2-tailed)	.000	
	N	21	31

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between right maxillary and mandibular pulp volumes in males and females

Correlations (Males)

		p2	p3
p2	Pearson Correlation	1	.941**
	Sig. (2-tailed)		.000
	N	23	21
p3	Pearson Correlation	.941**	1
	Sig. (2-tailed)	.000	
	N	21	35

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations (Females)

		p2	p3
p2	Pearson Correlation	1	.845**
	Sig. (2-tailed)		.000
	N	30	25
p3	Pearson Correlation	.845**	1
	Sig. (2-tailed)	.000	
	N	25	30

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 4 Test–Retest reliability of maxillary and mandibular pulp volumes in males and females

Males **Females**

Correlation coefficient for left maxillary pulp volume in males and females

Males

Case Processing Summary

		N	%
Cases	Valid	25	65.8
	Excluded[a]	13	34.2
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.967[a]	.927	.985	60.110	24	24	.000
Average Measures	.983	.962	.993	60.110	24	24	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Females

Case Processing Summary

		N	%
Cases	Valid	27	73.0
	Excluded[a]	10	27.0
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.970[a]	.934	.906	64.635	26	26	.000
Average Measures	.985	.966	.993	64.635	26	26	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for right maxillary pulp volume in males and females

Males

Case Processing Summary

		N	%
Cases	Valid	23	60.5
	Excluded[a]	15	39.5
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.951[a]	.888	.979	39.665	22	22	.000
Average Measures	.975	.941	.989	39.665	22	22	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Females

Case Processing Summary

		N	%
Cases	Valid	30	81.1
	Excluded[a]	7	18.9
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.933[a]	.864	.968	28.846	29	29	.000
Average Measures	.965	.927	.983	28.846	29	29	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for left mandibular pulp volume in males and females

Case Processing Summary

		N	%
Cases	Valid	35	92.1
	Excluded[a]	3	7.9
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Case Processing Summary

		N	%
Cases	Valid	30	81.1
	Excluded[a]	7	18.9
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.951[a]	.906	.975	40.041	34	34	.000
Average Measures	.975	.951	.987	40.041	34	34	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.965[a]	.928	.983	56.625	29	29	.000
Average Measures	.982	.963	.992	56.625	29	29	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for right mandibular pulp volume in males and females

Case Processing Summary

		N	%
Cases	Valid	34	89.5
	Excluded[a]	4	10.5
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Case Processing Summary

		N	%
Cases	Valid	31	83.8
	Excluded[a]	6	16.2
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.946[a]	.894	.972	35.797	33	33	.000
Average Measures	.972	.944	.986	35.797	33	33	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.968[a]	.934	.984	61.110	30	30	.000
Average Measures	.984	.966	.992	61.110	30	30	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Appendix 5 Inter–rater reliability of maxillary and mandibular pulp volumes in males and females

Correlation coefficient values for left maxillary pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	23	74.2
	Excluded[a]	8	25.8
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.981[a]	.955	.992	102.240	22	22	.000
Average Measures	.990	.977	.996	102.240	22	22	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for right maxillary pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	25	80.6
	Excluded[a]	6	19.4
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.980[a]	.956	.991	100.347	24	24	.000
Average Measures	.990	.977	.996	100.347	24	24	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for left mandibular pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	31	100.0
	Excluded[a]	0	.0
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.937[a]	.873	.969	30.551	30	30	.000
Average Measures	.967	.932	.984	30.551	30	30	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for right mandibular pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	29	93.5
	Excluded[a]	2	6.5
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.922[a]	.841	.963	24.737	28	28	.000
Average Measures	.960	.914	.981	24.737	28	28	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Appendix 6: Slice Interval scores between 0.3mm and 0.5 mm of tooth in males and females

Maxillary right teeth of males and females

Case Processing Summary

Gender			N	%
Male	Cases	Valid	12	80.0
		Excluded[a]	3	20.0
		Total	15	100.0
Female	Cases	Valid	12	80.0
		Excluded[a]	3	20.0
		Total	15	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

Gender		Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
			Lower Bound	Upper Bound	Value	df1	df2	Sig
Male	Single Measures	.974[a]	.911	.992	74.813	11	11	.000
	Average Measures	.987	.954	.996	74.813	11	11	.000
Female	Single Measures	.981[a]	.936	.995	105.064	11	11	.000
	Average Measures	.990	.967	.997	105.064	11	11	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Maxillary Left tooth males and females

Case Processing Summary

Gender			N	%
Male	Cases	Valid	14	93.3
		Excluded[a]	1	6.7
		Total	15	100.0
Female	Cases	Valid	11	73.3
		Excluded[a]	4	26.7
		Total	15	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

Gender		Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
			Lower Bound	Upper Bound	Value	df1	df2	Sig
Male	Single Measures	.962[a]	.887	.988	52.001	13	13	.000
	Average Measures	.981	.940	.994	52.001	13	13	.000
Female	Single Measures	.988[a]	.956	.997	163.332	10	10	.000
	Average Measures	.994	.977	.998	163.332	10	10	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Mandibular left canine tooth males and females

Case Processing Summary

Gender			N	%
Male	Cases	Valid	12	80.0
		Excluded[a]	3	20.0
		Total	15	100.0
Female	Cases	Valid	13	86.7
		Excluded[a]	2	13.3
		Total	15	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

Gender		Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
			Lower Bound	Upper Bound	Value	df1	df2	Sig
Male	Single Measures	.985[a]	.948	.996	131.146	11	11	.000
	Average Measures	.992	.974	.998	131.146	11	11	.000
Female	Single Measures	.989[a]	.964	.997	180.110	12	12	.000
	Average Measures	.994	.982	.998	180.110	12	12	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Mandibular Right canine tooth males and females

Case Processing Summary

Gender			N	%
Male	Cases	Valid	12	80.0
		Excluded[a]	3	20.0
		Total	15	100.0
Female	Cases	Valid	13	86.7
		Excluded[a]	2	13.3
		Total	15	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

Gender		Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
			Lower Bound	Upper Bound	Value	df1	df2	Sig
Male	Single Measures	.985[a]	.949	.996	132.735	11	11	.000
	Average Measures	.992	.974	.998	132.735	11	11	.000
Female	Single Measures	.986[a]	.956	.996	146.381	12	12	.000
	Average Measures	.993	.978	.998	146.381	12	12	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Appendix 7: Correlation scores of maxillary left and right, mandibular left and right, maxillary left and mandibular left, maxillary right and mandibular right canine tooth volumes in males and females

Males | **Females**

Pearson Correlation between maxillary left and right tooth volumes in males and females

Males – Correlations

		t1	t2
t1	Pearson Correlation	1	.961**
	Sig. (2-tailed)		.000
	N	25	20
t2	Pearson Correlation	.961**	1
	Sig. (2-tailed)	.000	
	N	20	23

**. Correlation is significant at the 0.01 level (2-tailed).

Females – Correlations

		t1	t2
t1	Pearson Correlation	1	.966**
	Sig. (2-tailed)		.000
	N	27	24
t2	Pearson Correlation	.966**	1
	Sig. (2-tailed)	.000	
	N	24	30

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between mandibular left and right tooth volumes in males and females

Males – Correlations

		t3	t4
t3	Pearson Correlation	1	.947**
	Sig. (2-tailed)		.000
	N	35	33
t4	Pearson Correlation	.947**	1
	Sig. (2-tailed)	.000	
	N	33	34

**. Correlation is significant at the 0.01 level (2-tailed).

Females – Correlations

		t3	t4
t3	Pearson Correlation	1	.959**
	Sig. (2-tailed)		.000
	N	30	26
t4	Pearson Correlation	.959**	1
	Sig. (2-tailed)	.000	
	N	26	31

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between left maxillary and mandibular tooth volumes in males and females

Males – Correlations

		t1	t4
t1	Pearson Correlation	1	.931**
	Sig. (2-tailed)		.000
	N	25	22
t4	Pearson Correlation	.931**	1
	Sig. (2-tailed)	.000	
	N	22	34

**. Correlation is significant at the 0.01 level (2-tailed).

Females – Correlations

		t1	t4
t1	Pearson Correlation	1	.907**
	Sig. (2-tailed)		.000
	N	27	21
t4	Pearson Correlation	.907**	1
	Sig. (2-tailed)	.000	
	N	21	31

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation between right maxillary and mandibular tooth volumes in males and females

Males – Correlations

		t2	t3
t2	Pearson Correlation	1	.959**
	Sig. (2-tailed)		.000
	N	23	21
t3	Pearson Correlation	.959**	1
	Sig. (2-tailed)	.000	
	N	21	35

**. Correlation is significant at the 0.01 level (2-tailed).

Females – Correlations

		t2	t3
t2	Pearson Correlation	1	.871**
	Sig. (2-tailed)		.000
	N	30	25
t3	Pearson Correlation	.071**	1
	Sig. (2-tailed)	.000	
	N	25	30

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 8: Test- Retest reliability of maxillary and mandibular teeth volumes in males and females

Males **Females**

Correlation coefficient for left maxillary tooth volume in males and females

Males

Case Processing Summary

		N	%
Cases	Valid	25	65.8
	Excluded[a]	13	34.2
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.979[a]	.952	.991	92.779	24	24	.000
Average Measures	.989	.976	.995	92.779	24	24	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Females

Case Processing Summary

		N	%
Cases	Valid	27	73.0
	Excluded[a]	10	27.0
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.978[a]	.952	.990	89.047	26	26	.000
Average Measures	.989	.975	.995	89.047	26	26	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for right maxillary tooth volume in males and females

Males

Case Processing Summary

		N	%
Cases	Valid	23	60.5
	Excluded[a]	15	39.5
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.983[a]	.961	.993	117.765	22	22	.000
Average Measures	.992	.980	.996	117.765	22	22	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Females

Case Processing Summary

		N	%
Cases	Valid	30	81.1
	Excluded[a]	7	18.9
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.983[a]	.966	.992	120.042	29	29	.000
Average Measures	.992	.982	.996	120.042	29	29	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for left mandibular tooth volume in males and females

Case Processing Summary

		N	%
Cases	Valid	35	92.1
	Excluded[a]	3	7.9
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Case Processing Summary

		N	%
Cases	Valid	30	81.1
	Excluded[a]	7	18.9
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.981[a]	.963	.990	105.096	34	34	.000
Average Measures	.990	.981	.995	105.096	34	34	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.980[a]	.958	.990	97.537	29	29	.000
Average Measures	.990	.978	.995	97.537	29	29	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient for right mandibular tooth volume in males and females

Case Processing Summary

		N	%
Cases	Valid	34	89.5
	Excluded[a]	4	10.5
	Total	38	100.0

a. Listwise deletion based on all variables in the procedure.

Case Processing Summary

		N	%
Cases	Valid	31	83.8
	Excluded[a]	6	16.2
	Total	37	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.984[a]	.968	.992	121.958	33	33	.000
Average Measures	.992	.984	.996	121.958	33	33	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.986[a]	.971	.993	143.439	30	30	.000
Average Measures	.993	.986	.997	143.439	30	30	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Appendix 9: Inter–rater reliability of maxillary and mandibular teeth volumes in males and females

Correlation coefficient values for left maxillary pulp volumes in males and females

Males

Case Processing Summary

		N	%
Cases	Valid	23	74.2
	Excluded[a]	8	25.8
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Females

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.985[a]	.964	.993	128.214	22	22	.000
Average Measures	.992	.982	.997	128.214	22	22	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for right maxillary pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	25	80.6
	Excluded[a]	6	19.4
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.880[a]	.746	.945	15.626	24	24	.000
Average Measures	.936	.855	.972	15.626	24	24	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for left mandibular pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	31	100.0
	Excluded[a]	0	.0
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.931[a]	.862	.966	27.889	30	30	.000
Average Measures	.964	.926	.983	27.889	30	30	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Correlation coefficient values for right mandibular pulp volumes in males and females

Case Processing Summary

		N	%
Cases	Valid	29	93.5
	Excluded[a]	2	6.5
	Total	31	100.0

a. Listwise deletion based on all variables in the procedure.

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.956[a]	.908	.979	44.359	28	28	.000
Average Measures	.977	.952	.989	44.359	28	28	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

Appendix 10: Publication

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ORIGINAL ARTICLE

Check for updates

Age estimation using canine pulp volumes in adults: a CBCT image analysis

Shakeel Kazmi [1] · Scheila Mânica [1] · Gavin Revie [1] · Simon Shepherd [1] · Mark Hector [1]

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Abstract

Secondary dentine deposition is responsible for the decrease in the volume of the pulp cavity with age. Therefore, the volume of the pulp cavity can be considered as a predictor for estimating age. The aims of this study were to investigate the relationship strength between canine pulp volumes and chronological age from homogenous (approximately equal numbers of individuals in each age range) age distribution and to assess the effect of sex as predictor in age estimation. This study was performed on 719 subjects of Pakistani origin. Cone beam computed tomography images of 521 left maxillary and 681 left mandibular canines were collected from 368 females and 349 males aged from 15 to 65 years. Planmeca Romexis® software was used to trace the outline of the pulp cavity and to calculate pulp volumes. Regression analysis was performed to assess the correlation between pulp volumes considering with and without sex as a predictor with chronological age. The obtained results showed that mandibular canine pulp volume and sex have the highest predictive power ($R^2 = 0.33$). The relationship between mandibular canine pulp volume and sex with chronological age demonstrates an odd S-shaped non-linear relationship. A statistically significant difference in volumes of pulp was found ($p = 0.000$) between males and females. The conclusion was that predictions using the pulp volume of the mandibular canine and sex produced the best estimates of chronological age.

Keywords Forensic odontology · Canine pulp volumes · Age estimation · Homogenous age distribution · Cone beam computed tomography

Introduction

Teeth are preferred in age estimation methods because they are less influenced by nutritional, hormonal, and environmental factors than bone [1–3]. Dental age estimation methods which rely on patterns of the tooth development and eruption have historically proven useful for estimating the chronological age in deciduous and permanent dentitions [4–6]. Tooth development and eruption is a reliable indicator for estimating age up to the age of 16 years. From 16 to 24 years, only third molar development can be assessed but there is significant variability, the accuracy is controversial and third molars are not always present [7].

When the tooth eruption is complete, secondary dentine deposition commences and the size of the pulp cavity decreases with age. This correlation was first investigated by Bodecker in 1925 [8]. In 1952, Gustafson introduced an invasive method for age estimation based on six age-related changes, including secondary dentine [9]. Further, non-invasive studies used conventional dental radiographs of tooth to measure the pulp-tooth linear measurements and area ratios to estimate the age [10, 11]. The results by Kvaal et al. revealed that pulp-tooth linear measurements ratio were the best correlated with age with an r^2 ranging from 0.56 to 0.76 [10]. Cameriere et al. introduced a method for estimating age based on pulp-tooth area ratio and obtained a value of (r^2) 0.85 [11]. These two methodologies are reproducible and non-destructive in nature therefore applied to different populations [12–16]. Permanent canines have been used in many studies because these teeth have larger pulp dimensions, subject to less wear from diet and demonstrate high level of survival compared with other teeth in dentition [3, 17–19].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00414-019-02147-5) contains supplementary material, which is available to authorized users.

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